CLINICAL STUDY PROTOCOL

GZ-2016-11621

A Pilot Randomized Study to Assess the Effect and Safety Profile of Thymoglobulin® in Primary Cardiac Transplant Recipients: A 12-month, multi-center, randomized, open-label study of efficacy comparing immediate treatment with and without Thymoglobulin® 1.5 mg/kg/d for 5 consecutive days in heart transplant recipients.

Amendment 8.0

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Protocol Summary

Title	A Pilot Randomized Study to Assess the Effect and Safety Profile of Thymoglobulin® in Primary Cardiac Transplant Recipients: A 12-month, multi center, randomized open-label study of efficacy comparing immediate treatment with and without Thymoglobulin® 1.5mg/kg/d for 5 consecutive days in heart transplant recipients
Primary Objectives	To describe between treatment groups the incidence of: • the composite primary endpoint of the development of de novo donor specific antibodies and ischemia on endomyocardial biopsy at 12 months post-transplantation.
Secondary Objectives	To describe between treatment groups: • Number of patients who develop cardiac allograft vasculopathy (CAV) (defined as a change ≥0.5mm in maximal intimal thickness (MIT) of the coronary arteries by intravascular ultrasound at 12 months as compared to baseline) • Correlations between significant changes in immune cell profiles and circulating antibodies., and their relation to any significant differences in clinical outcomes • Number of patients who experience acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within first 12 months after transplantation • Number of patients with biopsy proven cellular rejection ≥2R, biopsy proven antibody mediated rejection ≥AMR1 and any-treated rejection at 12 months post-transplantation • Number of acute cellular, antibody-mediated, hemodynamic compromise and any-treated rejection episodes per patient within the first 12 months post-transplantation • Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection by the ISHLT biopsy grading scale in the first 12 months post-transplantation • Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within the first 12 months • the incidence of Primary Graft Dysfunction (PGD) in the first 24 hours post-transplant • Patient and graft survival at 12 months post-transplantation • The types and number of patients with both fatal and non-fatal infectious complications (especially CMV infection) within the first 12 months post-transplantation • Freedom from the development of circulating antibodies within the first 12 months post transplantation, where circulating antibodies include donor specific antibodies (DSA), non-specific antibodies, and non-human leukocyte antigen antibodies • Change in coronary maximal intimal thickness, intimal area, intimal volume, vessel area, intimal index and percent atheroma volume (PAV) at matched sites by intravascular ultrasound at 12 months • Maintenance doses of mycophenolate mofetil, tacrolimus, sirolimus, and cumulative dose of corticosteroids at 12 months post-transplantation • Number of hospital days per patient, both during the transplant period and during the post-transplant period at 3 months, 6 months, and 1 year

	• Number of patients requiring hospitalization by 3 months, by 6 months, or by 1 year post-transplantation
Study Design	Randomized and controlled, descriptive study
Sample Size	60 primary cardiac transplant recipients
Number of Centers	Two centers
Selection Criteria	• Primary cardiac transplantation • 18 to 74 years old • Men and non-pregnant women
Study Medication	Thymoglobulin® (rabbit anti-thymocyte globulin 25mg/5mL vials, IV)
Comparator	No induction therapy
Test Dose/Duration	• 1.5 mg/kg/24 hours X 5 doses I.V. • First dose administered within 24 hours after transplant if subject is randomized to receive Thymoglobulin. • Total duration 5 doses over 5 days
Route of Administration	Intravenous— 0.5mg/ml IV solution in D5W or NS. The First two doses infused over 8 hours. All subsequent doses infused over 4-8 hours
Primary Parameters of Efficacy	• Incidence of the primary endpoint of the development of de novo donor specific antibodies and ischemia on endomyocardial biopsy at 12 months post-transplant
Secondary Parameters of Efficacy	To describe between groups: • Changes in percentages of various subsets of immune cells at pre transplant, 3 months, and 6 months. Changes in percentages of various circulating antibodies at pre-transplant, 1 month, 3 months, 6 months, and 12 months after transplant. • Freedom from cardiac allograft vasculopathy (CAV) (defined as a change ≥0.5mm in maximal intimal thickness (MIT) of the coronary arteries by intravascular ultrasound at 12 months as compared to baseline).Change in coronary maximal intimal thickness intimal area, intimal volume, vessel area, intimal index, and percent atheroma volume at matched sites by intravascular ultrasound at 12 months Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within first 12 months after transplantation. • Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection episodes per patient within the first 12 months post-transplantation • Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection by the ISHLT biopsy grading scale in the first 12 months post-transplantation • Freedom from biopsy proven cellular rejection ≥2R, biopsy proven antibody mediated rejection ≥AMR1 and any-treated rejection at 12 months post-transplantation • Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within the first 12 months post-transplantation • Freedom from primary graft dysfunction (PGD) in the first 24 hours post-transplantation • Patient and graft survival at 12 months post-transplantation

	• Freedom from fatal and non-fatal infectious complications (especially CMV infection) within the first 12 months post-transplantation • Freedom from the development of circulating antibodies within the first 12 months post-transplantation • Average maintenance doses of Mycophenolate Mofetil, tacrolimus, and cumulative dose of corticosteroids at 12 months post-transplantation • Average number of hospital days per patient, both during the transplant period and during the post-transplant period at 3 months, 6 months, and 1 year, • Freedom from hospitalizations by 3 months, 6 months, or 12 months post-transplantation. • Freedom from the development of circulating antibodies
Main Parameters of Safety	• Number of patients with clinical CMV infection • Incidence of adverse events, including opportunistic infections, and malignancies • Changes in laboratory values, especially absolute total white blood cell, platelet, and hemoglobin counts
Randomization	Patients qualifying for the study will be randomized in a 1:1 ratio to either Thymoglobulin® or No Induction or Control during the immediate post transplantation period after confirming the eligibility criteria for randomization.
Duration	The study was initiated in September 2018 and enrollment will continue until October 31st, 2023. The study will be completed 24 months after the last patient has entered. Study completion is expected by October 31, 2025.

GLOSSARY OF ABBREVIATIONS

ABO	The three blood types—**A** type, **B** type, and **O** type
ACE	**A**ngiotensin **C**onverting **E**nzyme
AE	**A**dverse **E**vent
AMR	**A**ntibody-**M**ediated **R**ejection
ANOVA	**An**alysis **O**f **Va**riance
ATG	**A**nti-**T**hymocyte **G**lobulin (of rabbit unless otherwise indicated)
beta-HCG	**beta-H**uman **C**horionic **G**onadotropin
bid	taken twice a day
BUN	**B**lood **U**rea **N**itrogen
CAV	**C**ardiac **A**llograft **V**asculopathy
CBC	**C**omplete **B**lood **C**ount
CI	**C**ardiac **I**ndex
CMV	**C**yto**m**egalo**v**irus
CO2	**C**arbon **D**ioxide
CRF	**C**ase **R**eport **F**orm
EBV	**E**pstein **B**arr **V**irus
ECG	**E**lectro**c**ardio**g**ram
ELISA	**E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay
ESF	**E**ligibility **S**creening **F**orm
FDA	**F**ederal **D**rug **A**dministration
GGT	**G**amma **G**lutamate **T**ransferase
HDL	**H**igh-**D**ensity **L**ipoprotein
HIPAA	**H**ealth **I**nsurance **P**ortability and **A**ccountability **A**ct
HIV-1	**H**uman **I**mmunodeficiency **V**irus type **1**
HLA	**H**uman **L**eucocyte **A**ntigen
HMG CoA Reductase Inhibitor	3-**H**ydroxy-3-**M**ethyl-**G**lutaryl **Co**enzyme **A Reductase Inhibitor** (statins)
ICU	**I**ntensive **C**are **U**nit
IFN	**I**nter**f**ero**n**
IgG	**I**mmuno**g**lobulin **G**
IL	**I**nter**L**eukin (a cytokine)
IRI	**I**schemia-**R**eperfusion **I**njury
ISHLT	**I**nternational **S**ociety for **H**eart and **L**ung **T**ransplantation
IU	**I**nternational **U**nits
IV	**I**ntra**V**enous
IVUS	**I**ntra**v**ascular **U**ltra**s**ound
LDH	**L**actate **D**e**H**ydrogenase
LDL	**L**ow-**D**ensity **L**ipoprotein
LVAD	**L**eft **V**entricular **A**ssist **D**evice
MHC	**M**ajor **H**isto**C**ompatibility
MMF	**M**ycophenolate **M**o**F**etil
NIAID	**N**ational **I**nstitutes of **A**llergy and **I**nfectious **D**isease
OI	**O**pportunistic **I**nfections
PO/po	**P**er **O**rum, taken orally or by mouth
PBMC	**P**eripheral **B**lood **M**ononuclear **C**ells
PGD	**P**rimary **G**raft **D**ysfunction
PRA	**P**anel of **R**eactive **A**ntibodies

PRT	**P**anel of **R**eactive **T**-cells
PRBC	**P**acked **R**ed **B**lood **C**ells
PTLD	**P**ost-**T**ransplant **L**ymphoproliferative **D**isorder
qd	Taken/administered once a day
RBC	**R**ed **B**lood **C**ell Count
SAE	**S**evere **A**dverse **E**vents
SCID	**S**evere **C**ombined **I**mmuno**D**eficiency
SGOT	**S**erum **G**lutamate **O**xalacetate **T**ransaminase
SGPT	**S**erum **G**lutamate **P**yruvate **T**ransaminase
sirolimus	= rapamycin
tid	taken three times a day
TNF	**T**umor **N**ecrosis **F**actor (a cytokine)
VAD	**V**entricular **A**ssist **D**evice
WBC	**W**hite **B**lood **C**ell Count

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1. BACKGROUND

1.1 DISEASE BACKGROUND

Cardiac transplantation is currently the procedure of choice for selected patients with end-stage heart disease that is not amenable to further medical intervention or conventional cardiac procedures. Over the past 10 years, experience, research, and new drugs have together resulted in an increase in the median survival of cardiac transplant recipients to 11 years.[1] However, survival for transplant recipients is still limited by cardiac allograft vasculopathy (CAV), infectious complications, malignancy, and rejection. Reports of spontaneous allograft tolerance emphasize the importance of understanding how the immune system interacts with the allograft and how the immune system might be manipulated to achieve allograft tolerance. Described below is the effect of each of these factors on recipient survival and what is known about the effect of antithymocyte globulin (ATG/Thymoglobulin®) on each of these factors.

1.1.1 Donor Specific Antibodies

The development of donor-specific anti-HLA antibodies is an established risk factor for poor outcomes after heart transplantation. *De novo* DSA after heart transplant is associated with an increased risk of acute cellular rejection, antibody mediated rejection and CAV.[2] In a retrospective, single-center study of 243 patients who underwent heart transplantation between 1995 and 2004, 57 patients developed *de novo* DSA. Univariate analysis found that *de novo* and persistent DSA (found in two consecutive samples) were associated with increased mortality (HR 3.198, p = 0.0018; HR 4.351, p = 0.002). Similarly, on multivariate analysis, *de novo*, persistent DSA was strongly predictive of mortality (HR 4.331, p <0.0004). DSA were associated with biopsy proven rejection in the first year after heart transplant.[3] A large retrospective study of 950 heart transplant patients examining the effects of *de novo* DSA found that survival was 52% for patients developing *de novo* DSA in the first versus 70% in patients who developed no DSA at 15 years post-transplantation.[4] Another retrospective study examining a series of 71 heart transplant recipients, 7 of whom developed *de novo* DSA demonstrated an association with the development of CAV at 3 years post-transplant. Of the patients who developed CAV, 55% had Class II anti-HLA antibodies. The presence of these antibodies also correlated with death due to allograft failure.[5] A recent study examining biopsy-proven AMR and outcomes found that 71% of patients with AMR had DSA and the presence of DSA with AMR increased the odds of graft dysfunction (OR 5.37, 95% CI 1.34 to 21.47, p = 0.018).[6] Another recent study of 122 consecutive heart transplant patients assessed the impact of developing *de novo* DSA on a composite primary endpoint of death or graft dysfunction. During a median follow-up of 3.3 years, 28% of patients developed *de novo* DSA. Development of antibodies to HLA-DQ was associated with an increased risk of the primary endpoint (HR 6.15, 95% CI 2.57-14.75, p=0.001).[7]

1.1.2 Cardiac Allograft Vasculopathy

Cardiac allograft vasculopathy (CAV) accounts for a significant number of deaths in cardiac recipients after the first-year post-transplant. In a recent registry report from the ISHLT, CAV was noted in 7.8% of patients at 1 year post-transplant, 30% of patients at 5 years, and 50% at 10 years.[1] CAV is the third most common cause of death after 3 years post-transplant. CAV carries

a poor prognosis with a mortality rate of 50% within 2 years of diagnosis.[8] CAV may occur as early as one year after transplantation. Disease appearing early following transplantation is more aggressive and associated with a worse prognosis, with two thirds of patients suffering coronary events within 5 years following the detection of CAV.[9-12] In one study, those with angiographic disease had a relative risk of any cardiac event of 3.4 and a relative risk of death of 4.6 compared with those without disease.[9] Due to the denervated transplanted heart, cardiac ischemia or infarction does not typically present with chest pain. Patients with myocardial infarction frequently lack typical ECG changes due to either baseline abnormalities or to heterogeneous disease resulting from diffuse vasculopathy. In general, the absence of symptoms and ECG findings often lead to lower utilization of revascularization therapies and consequently, worse outcomes such as heart failure, arrhythmia or sudden death.[9]

With the exception of re-transplantation, available treatments for CAV only slow its progression. Because of the diffuse and concentric nature of CAV and its involvement in small vessels, percutaneous intervention and coronary artery bypass are not effective treatments. Medications which have been demonstrated to be efficacious in clinical trials include atorvastatin,[13] pravastatin,[14,15] simvastatin,[16] calcium channel blockers,[17-19] ACE inhibitors,[19] aspirin,[20] vitamin C and vitamin E,[21] ganciclovir (in CMV mismatch),[22] and possibly sirolimus[23] and everolimus.[24] Modifiable risk factors include smoking cessation, control of diabetes, and freedom from rejection.

Re-transplantation for CAV is reasonable as the 1-year actuarial survival approaches the 1-year survival following primary transplants. Patients having a second heart transplant do not have an increased risk for development of CAV in the second donor heart.[25,26] However, the scarcity of donor hearts both creates an ethical dilemma and limits re-transplantation to only a small minority of patients.

CAV occurs both in recipients who were transplanted for non-ischemic heart disease as well as those who were transplanted for ischemic heart disease. In contrast to the more focal lesions of conventional atherosclerosis, CAV exhibits a more diffuse nature in the form of concentric narrowing with frequent involvement of large and medium sized vessels as well as the microvasculature.[27] The disease involves only the allograft and spares the native arteries. Together these observations suggest an immunologic basis for CAV. This is also consistent with early experimental evidence where hearts transplanted into a genetically different recipient were affected by CAV and those transplanted into a genetically identical recipient were spared.

However, there are also non-immunological clinical risk factors for CAV, which have been consistently identified in a number of retrospective studies. These include preservation injury, the cause of donor death, donor graft ischemic time, older donor age, early rejection, the host allo-immune response, and cytomegalovirus infection[9]. Although not causative, the conventional risk factors for atherosclerosis, such as hypertension, hyperlipidemia, glucose intolerance, and oxidative stress, also accelerate the disease process. Although the non-immunological risk factors may seem diverse and un-related, one result in common is causing an increase in the susceptibility of the endothelium to damage. Therefore, currently it is believed that CAV results from an initial injury to the donor endothelium from ischemic events that occur pre- or peri- operatively.[28,29] This injury is followed by intimal hyperplasia and the proliferation of vascular smooth muscle cells, which is believed to reflect sustained activation of the recipient's immune system against and ongoing immune response against donor MHC antigens that is perpetuated by subsequent

episodes of allograft rejection.[30] This is evidenced by a study in which the T-cell alloresponses to synthetic peptides corresponding to the hypervariable region of the mismatched HLA-DR antigen of the donor were prospectively followed in 34 heart transplant recipients from 1-3 years posttransplant. Patients who developed CAV and who had more frequent rejection episodes (i.e. unable to develop tolerance) exhibited (a) a progressive diminution in the number of T-cell clones recognizing the immunodominant donor alloantigen determinants and a gradual shift in the T-cell repertoire, leading to the new recognition of multiple non-dominant epitopes, which in turn perpetuated the rejection process; (b) stronger T-cell alloreactivity during the first 6 months posttransplant; and (c) persistent T-cell alloreactivity after the first 6 months.[31]

Experimental and clinical findings demonstrate that both humoral and cellular immune responses contribute to the pathogenesis of graft vasculopathy (see Section 1.1.5 below on humoral and cellular rejection).[32] Evidence for humoral involvement in clinical heart transplantation comes from the observation that circulating anti-HLA antibodies and anti-endothelial cell surface antibodies have been associated with CAV. When compared with control patients, those patients who develop AMR and have a higher frequency of AMR progress to CAV earlier and at an increased frequency. The vast majority of patients who demonstrate histological AMR suffer from CAV by 5 years after transplantation regardless of whether they have concomitant hemodynamic compromise.[33] Animal studies support these observations. Russell *et al.* reported that hearts transplanted into B cell-deficient mice that are incapable of producing immunoglobulins develop only minor CAV.[34] Another animal study showed that hearts transplanted into immunodeficient SCID mice survived indefinitely with no significant vasculopathy. However, vascular lesions rich in macrophages and NK cells developed in the SCID mice upon treatment with an antiserum reactive with donor antigens.[32] In rodent models of allograft vasculopathy arterial lesions are observed to first develop as endothelitis and subsequently progress to smooth muscle cell-rich fibrosis.

Cellular rejection also plays a significant role in the development of CAV. In animal studies where knockout mice deficient in both cellular and humoral immunity or only humoral immunity were transplanted, CAV developed in knockout mice that were deficient only in humoral immunity, suggesting that T-cellular rejection does play a role in the development of CAV.[35] Russell, *et al.* also showed that the histological features of the CAV produced in SCID mice transfused with antiserum are distinct from those lesions in mice which have preserved cellular and humoral activities. T- cells, including CD4+, CD8+, and Mac-1+ cells, are seen in the arterial lesions of the mice with preserved cellular and humoral immunity.[36] These cells secrete a myriad of inflammatory markers, including cell adhesion molecules, cytokines, chemokines, growth factors, and macrophage activators.[37] In another study depletion of CD4+ T-cells (but not CD8+ T-cells) prevents arterial lesion formation in these rodent models.[38] In addition, interferon-gamma and transcription factor stat 4 knockout animals (inflammatory cytokine products produced by TH1 cells) demonstrated decreased vasculopathy.

The role of **antibodies and AMR in the development of CAV** is equivocal. Evidence from animal models of CAV have demonstrated that induction therapy that depleted B cells is associated with less severe CAV development.[39] Whether induction therapy can prevent the development of AMR is human subjects remains to be proven. A recent study examined 40 explanted hearts and 402 endomyocardial biopsy (EMB) samples from these hearts before graft loss.[40] Their work demonstrated that 47.5% of explanted, failing allografts had undiagnosed antibody mediated rejection (AMR) years earlier. Immune modulation targeting the humoral response could,

therefore, play a role in preventing or slowing the progression of CAV. These finding were in agreement with an earlier 5-year study examining the freedom from CAV in heart transplant recipients with asymptomatic and untreated AMR.[41] CAV more frequently developed in patients with asymptomatic and untreated AMR than in controls. Another study examining 71 hear transplant recipients demonstrated that antibodies against class II HLA were strongly associated with the development of CAV by 3-years post transplantation.[5]

Whether antibody induction therapy affects the development of CAV remains controversial. Thus far, controlled studies of monoclonal antibodies have not demonstrated a beneficial outcome in either CAV or rejection. However, the role of ATG, a polyclonal antibody preparation, remains controversial. In contrast to the monoclonal antibodies, ATG, being a polyclonal agent, is able to target all of the potential mechanisms that contribute to the development of CAV—T-cell activation, B-cell activation, antibody formation, induction of tolerogenic cells, and modulation of lymphocyte-endothelium interactions. These mechanisms will be discussed in more detail in the sections to follow. Several small retrospective studies have shown that the incidence of CAV in cardiac transplant recipients who received ATG induction therapy is significantly lower.[42-44] Therefore, ATG induction may prevent the development of CAV and its mechanism may be related to its polyclonal nature.

1.1.3 Infection in Transplant Recipients

Immunosuppression using triple drug therapy is currently the mainstay of prevention against cardiac allograft rejection. However, while too little immunosuppression increases the risk for rejection, too much immunosuppression increases the risk for infection. Currently there are no diagnostic tests that can guide the amount of immunosuppression needed to achieve a reasonable balance between rejection and infection. The amount of immunosuppression needed to prevent both infection and rejection is highly individual. Therefore despite the fact that newer immunosuppressive regimens have resulted in a trend towards a lower rejection rate in the United States, about 50% of cardiac transplant recipients are treated for infection within the first year after transplant.[45]

Cardiac allograft recipients are prone to acquiring persistent bacterial, fungal, and viral infections. Infections within the first month post-transplant are usually nosocomial or donor-derived. Between one and six months post-transplant, levels of immunosuppression usually higher and thus the most common infections are due to the activation of latent infection within the recipient or opportunistic. Infections acquired greater than six months post-transplant are usually community-acquired. **The incidence and severity of post-transplant infections has been significantly altered in the past decade by a better understanding of the risk factors for infection from research, implementation of anti-microbial prophylaxes and vaccinations both preemptively and universally, and improved methods for the detection of infection.**

Studies have demonstrated that changes to immunosuppression regimens either by the introduction of a new drug or the addition/subtraction of a drug can result in new patterns of infection. For example, the use of azathioprine is associated with a lower rate of bacterial, fungal, and aspergillus infections than use of proliferation signal inhibitors (PSI) such as everolimus or sirolimus, but the incidence of viral infection is significantly reduced in patients receiving PSI.[24,46] In a study that compared everolimus to traditional immunosuppressant treatment, viral infections occurred in 31.3% of subjects receiving azathioprine versus 14.8% and 17.1% for everolimus 1.5

mg/day and 3.0 mg/day groups, respectively (*P*<0.001). Use of everolimus reduced the risk of CMV infection by almost one-third as compared with azathioprine-based therapy.[24]

Whether induction therapy with ATG results in an increased risk of infection when used concomitantly with standardized triple immune suppression therapy in cardiac transplant recipients remains controversial as a randomized controlled trial has not yet been performed that addresses this question. Unfortunately, the initial studies of ATG induction therapy used to obtain FDA approval in renal transplant recipients cannot be applied to cardiac transplant recipients. Even though the immunosuppressive drugs studied in combination with ATG were the same as those used routinely after cardiac transplantation, ATG was administered early after transplantation to the delay the initiation of the nephrotoxic immunosuppressants, whereas in cardiac transplantation there is usually no delay in the administration of immunosuppressive drugs. However, **there is evidence to suggest that the concurrent administration of ATG with the initiation of routine triple immunosuppressive drug therapy may be safe. Concomitant administration of ATG with corticosteroids, azathioprine, and cyclosporine in pediatric heart transplant recipients was not found to result in an increased rate of infection.[47] Furthermore, study of ATG induction given at the same time with corticosteroids, mycophenolate mofetil, and tacrolimus in adolescents undergoing renal transplantation also did not result in an increased rate of infection.[48]**

1.1.4 Post-transplant Lymphoproliferative Disease in Transplant Recipients

Post-transplant lymphoproliferative diseases (PTLD) are another adverse event unique to transplant and chronic immunosuppression**. PTLD is the most common malignancy found in the first-year post-transplant and this is thought to be due to higher immunosuppression during this period.** PTLD is a type of lymphoma associated with Epstein-Barr virus (EBV), heightened immunosuppression, and chronic antigenic stimulation. As PTLD consists of a heterogeneous group of disorders, the treatment regimen cannot be standardized. Currently, modalities used in the treatment of PTLD consist of reduction of immunosuppression, radiation, surgical excision, monoclonal antibodies, interferon-alfa, and chemotherapy.

Whether ATG is associated with increased risk for PTLD remains controversial. Again, a controlled trial has not yet been performed, and so far the largest retrospective study conducted on this topic, which was based on the Scientific Registry of Transplant Recipients (SRTR), did not show any difference in the relative risk of developing PTLD between any specific lymphocyte-depleting antibodies.[49]

1.1.5 Graft Tolerance

Transplant tolerance is defined as full acceptance of the allograft in the absence of chronic immunosuppression while maintaining appropriate responsiveness to pathogens.[50] A growing body of literature supports the existence of distinct subsets of cells within each arm of the immune system that play a role in both inducing and maintaining transplant tolerance *in vivo*. These regulatory cells fall into three main categories—T-regulatory cells (T-regs), T suppressor cells, and dendritic cells.[51] T-regs are generally CD4+CD25+ and can inhibit the activity of dendritic cells, natural killer cells, and activated T and B cells.[51] T suppressors inhibit the immune system by rendering antigen presenting cells tolerogenic, producing immunosuppressive cytokines such as TGFß and IL-10, and inhibiting the activation of CD4+ and CD8+ effector memory cells, like T-

reg.[50] Dendritic cells (DC) are another arm of the immune system which can mediate both rejection and tolerance in transplantation. The milieu in which the DC interact with T-cells can determine whether the immune system decides to reject or tolerate the organ. Mature DC promote adaptive immunity and the activation of allogeneic T-cells, whereas immature DC are thought to promote tolerance.[50]

Historically it was thought that the underlying molecular mechanism of ATG was due to an overwhelming depletion of T-cells that then resulted in an inability of the host's immune system to react with the donor graft. However, **in recent years human and animal studies have demonstrated the potential for ATG to generate tolerance.**

ATG has been shown to selectively delete activated T-cells and activated B-cells, to inhibit cytokine release by dendritic cells and activated T-cells, and to modulate adhesion and cell-trafficking molecules, thereby suppressing the activation of key early host immune responses to the graft.[50] In addition to these mechanisms there is evidence that ATG can also promote the phenotype and expansion of regulatory T-cells and immature DC. Two studies have demonstrated that *in vitro* treatment of lymphocytes with ATG caused the selective expansion of T-regs by promoting the conversion of CD4+CD25- cells to CD4+CD25+ cells followed by rapid proliferation.[52] These ATG-generated T-reg are capable of suppressing the immune responses of activated lymphocytes *in vitro.*[52,53] Furthermore, adoptive transfer of ATG-treated lymphocytes into animal models of graft versus host disease caused increased survival and this effect appeared to be from the inhibition of allogeneic CD8+ T-cell expansion *in vivo.*[52] ATG also promotes the expansion of the natural killer regulatory T-cells (NK-T). Mice pre-treated with total lymphoid irradiation (TLI) and ATG have significantly improved survival after bone marrow transplant. The effect is attributed to NK-T-cells because the effect of TLI/ATG pre-treatment is absent in mice that are NK-T deficient.[50]

Lastly, in support of ATG's tolerogenic properties is the recent case report of a renal transplant recipient who received TLI/ATG pre-conditioning before bone marrow and renal transplantation at Stanford. This patient has demonstrated any evidence of graft rejection or graft-versus-host disease and has been immunosuppressant-free for 28 months.[54]

1.1.6 Graft Rejection

Despite the significant advances made in the past decade, **graft rejection remains a significant barrier to the survival and long term outcomes of these patients.[55] Currently, some centers report at least one episode of high grade acute cellular rejection during the first year following transplantation in up to 45% of cardiac allograft recipients.[56] Approximately 20% of all recipients experience AMR at some time post-transplantation and this percentage has not been positively influenced by the therapies that have been effective in reducing the incidence of cellular rejection.[57]**

The consequences of acute and chronic rejection are donor graft dysfunction, graft failure, and the development of CAV, respectively, that may result in death or re-transplantation. The acute hemodynamic compromise may not resolve even with prompt and successful medical intervention. With multiple episodes of rejection or chronic rejection, the donor organ becomes progressively fibrotic and restrictive in physiology with deterioration in systolic function. A rejection episode within the first month of cardiac transplant is an independent risk factor of death.[58] Patients experiencing one or more clinical rejection episode that requires steroid or

antibody therapy during the first month after transplantation are also under a significantly higher risk of developing recurrent rejection within two months as well as death from recurrent rejection.[58] Multiple studies have shown that recurrent cellular and AMR episodes are associated with an increased risk of CAV, which is currently the major cause of late death in cardiac recipients. In fact those patients who have accumulated more than 0.75 rejections per year have the highest risk for the development of CAV[29] (see Section 1.1.2 for discussion of CAV). The secondary negative consequences of rejection are related to treatment. Rejection episodes are usually initially treated with high dose pulse steroids and increased immunosuppression, which predisposes the recipients to increased morbidity due to the side effects of these agents, such as opportunistic infections, malignancies, renal failure, bone loss, and glucose intolerance.

Rejection can be classified as either hyperacute, cellular, and humoral. Hyperacute rejection of a transplanted organ is due to the presence of pre-formed antibodies and has been greatly reduced by preoperative screening for alloreactive antibodies and prospective, donor-specific crossmatching in sensitized recipients. Nowadays, hyperacute rejection is an extremely rare event.[59] However, acute cellular rejection remains a significant problem. **Acute cellular rejection is the most common form of rejection, occurring days to months after transplantation and is responsible for the loss of 10-20% of allografts.[55]** The activated T-lymphocyte plays a major role in the immune cascade that leads to (1) the recruitment and proliferation of various cell lines, including macrophages, B cells, helper T-cells, cytotoxic T-cells, natural killer cells, etc., (2) the secretion of various cytokines (TNF-alpha, IFN-gamma, IL-2, IL-4, IL-5, and IL-6) and (3) the subsequent damage to donor endothelium and myocytes, which pathologically manifests as cellular rejection.[59]

AMR in cardiac transplantation is currently thought to represent the consequences of antibody-induced and complement-mediated activation of endothelial cells, secretion of cytokines, increased endothelial cell adherence of leucocytes and subsequent ischemic damage to the graft.[59] Histologically, AMR in a biopsy specimen is defined by the presence of deposits in a vascular pattern that are comprised of immunoglobulin, complement, and fibrinogen deposits as visualized by immunofluoresence; endothelial cell swelling, distending capillaries, hemorrhage, and interstitial swelling as visualized by light microscopy; and positive immunoperoxidase staining of paraffin-embedded tissue.[33,60] The vascular deposits are hypothesized to represent immune complexes from a number of mechanisms, including the formation of host cytotoxic antibodies against donor MHC Class II antigens (HLA-DR) found on donor endothelium; and anti-endothelial cell antibody generation. Clinically, patients with AMR have an increased risk of fatal irreversible rejection with acute hemodynamic compromise, the development of accelerated CAV, and death.[33,60]

The mainstay of therapy is the prevention of rejection by the initiation and maintenance of chronic immunosuppression using triple drug therapy soon after transplantation. Most centers utilize steroids in combination with two other immunosuppressive agents, usually a calcineurin inhibitor (cyclosporine or tacrolimus) with a purine synthesis inhibitor (azathioprine or mycophenolate mofetil). Steroids reduce the transcription of many genes involved in inflammation and immunity by suppressing the activation of the transcriptional regulator nuclear factor kappa (NF-kB) and enhancing the expression of inhibitory factor intracellular kappa B (IkB).[61] Cyclosporine (also known as Sandimmune and Neoral) and tacrolimus both inhibit the function of calcineurin, thus blocking the upregulation of IL-2 transcription and subsequently preventing IL-2 production and activation of the T-cell. Historically the advent of cyclosporine in

the 1980s accounts for the re-emergence of heart transplantation, which was prohibited and abandoned in the previous decade due to high rejection and mortality rates. Besides the lower incidence of side effects such as gingival hypertrophy, hypertension, and hirsutism, tacrolimus is now favored over cyclosporine because of two trials in which tacrolimus treated heart transplant patients had significantly lower rejection rates as compared to cyclosporine treated groups.[59] Azathioprine (also known as Imuran) and mycophenolate mofetil (also known as MMF) inhibit the proliferation of lymphocytes by interfering with purine synthesis. The target of MMF, the enzyme IMPDH (inosine monophosphate dehydrogenase), is a unique step in the *de novo* pathway of purine synthesis in lymphocytes, which makes MMF more lymphocyte specific than azathioprine.[59] MMF affects not only T lymphocyte activity, but is also associated with a reduction of B lymphocytes and a downregulation of activation markers on B cells.[62] A number of comparison trials suggested that MMF is more efficacious than azathioprine in reducing severe rejection and 1 year mortality in heart transplant recipients, resulting in the increased utilization of MMF over azathioprine.[59] Besides their lipid lowering effects, statins also have anti-inflammatory and immunosuppressive properties and have been shown to be associated with rejection prevention in heart transplant recipients.[14-16] Pravastatin, simvastatin, and atorvastatin are now routinely used in cardiac recipients. Doses are titrated to patient tolerance rather than to an absolute lipid level.

As already mentioned above, currently the main mode of therapy for acute rejection is high dose pulse steroids. Acute rejection that is refractory to steroids may be treated with a repeat course of high dose pulse steroids, monoclonal antibodies, methotrexate, rabbit-antithymocyte globulin, total lymphoid irradiation, photopheresis, or plasmapheresis, depending on the protocol of the institution**. Immunosuppression protocols vary among institutions because there are very few prospective, randomized trials in heart transplant recipients.** Likewise, perioperative induction therapy with monoclonal or polyclonal antibodies is also subject to the protocol of each individual transplant center and is recently estimated to be used in approximately 47% of cardiac allograft recipients world-wide.[55] Daclizumab and basiliximab are both monoclonal antibodies directed against the IL-2 receptor (IL-2R) and due to increased mortality seen in a randomized, double blinded, No Induction or Control controlled trial (unpublished data), daclizumab has fallen out of use.

Multiple studies of antibody induction therapy, both prospective and retrospective have shown either superior or at least equivocal results of the polyclonal antibodies over monoclonal antibodies with better short and long term safety profiles.[63-66] However, **whether induction therapy using polyclonal antibodies can prevent rejection in cardiac transplant recipients has yet to be definitively demonstrated.** Similar to CAV, the effect of ATG induction on rejection has been studied in several retrospective, uncontrolled studies. The majority of these studies have demonstrated that ATG induction therapy prevents rejection in cardiac transplant recipients.[21,42,67]

1.1.7 Ischemia-reperfusion injury, primary graft dysfunction, and significance to CAV and ATG

Two basic mechanisms play an important role in IRI: (a) systemic imbalance of oxidative stress/antioxidant status and (b) restoration of metabolic processes which trigger the immune/inflammatory responses.

It seems that reactive oxygen species (ROS) initiate and induce the adaptive alloimmune response (acute rejection) predominantly through activation of antigen-presenting cells. Furthermore, the ROS-induced injury contributes to the development of atherosclerosis of donor heart vessels (chronic rejection) through endothelial injury-induced proliferation of smooth muscle cells.[68] Loss of oxygen supply during the ischemic period and subsequent reperfusion of the graft trigger the loss of osmotic equilibrium and increased permeability of cellular membranes, which leads to cell necrosis and decreased overall organ function.[68-73] Furthermore, the formation of reactive oxygen species that cause direct oxidative damage to nucleic acids, proteins, and lipids plays an important role in aggravating cell and tissue damage.[71,74,75] Tissue hypoxia is only one of the factors contributing to cellular damage related to ischemia-reperfusion during organ transplantation. Reperfusion also triggers the expression of inflammatory cytokines and adhesion molecules that increases the rate of apoptosis in the reperfused tissue.[69,76]

The role of white blood cells, which are closely related to the development of inflammatory damage in ischemia-reperfusion, has been demonstrated in various studies.[77-79] Preservation and revascularization which occur early in the transplantation process initiate a cascade of molecular and cellular events which trigger the release of proinflammatory mediators and attract various cell types which infiltrate the tissues.[70,78] Leukocytes have been considered to be responsible for many pathophysiologic changes during IRI.[80-86] They may exacerbate tissue hypoxia by plugging capillaries[77,86,87] and mediate direct cytotoxicity by producing oxygen radicals[88] and proteolytic enzymes.[84] The alteration of vascular resistance during ischemia-reperfusion is another important role of these mediators causing IRI.[79] In fact, activation of components of the inflammatory response exacerbates the damage already caused by the oxidative radicals.[85] Therefore, already existing ischemia-induced damage is further exacerbated by cytotoxic cells and effects on adhesion molecules.[76,89]

Given the pathological processes detailed above, ischemia-reperfusion injury has the clear effect of damage to cardiac tissue, with clinical consequences. In general, the risk of poor immediate post-transplant outcome directly correlates with the cold ischemia time. Prolonged ischemic time directly correlates with poorer 30-day survival[90] and is acknowledged as a risk factor for Primary Graft Dysfunction (PGD),[91] which itself has a high mortality rate.

The definition of PGD has been controversial in heart transplantation, but a standardized definition has emerged following an international consensus conference in April 2013.[92] PGD is defined by either left or right ventricular failure that occurs within 24 h of surgery. Severity is graded by the affected ventricle and the nature of circulatory support required. Survival rates are poor in patients with high grade PGD, with retrospective studies demonstrating a 30 day survival of as low as 14%.[93] The ISHLT registry indicates PGD occurs in 2-26% of heart transplants, although exact rates are unclear due to the aforementioned previous lack of standardized criteria.[94]

Table 6 Definition of Severity Scale for Primary Graft Dysfunction (PGD)

1. PGD-Left ventricle (PGD-LV):	*Mild PGD–LV*: *One* of the following criteria must be met:	LVEF $\leq$ 40% by echocardiography, *or* Hemodynamics with RAP $>$ 15 mm Hg, PCWP $>$ 20 mm Hg, CI $<$ 2.0 L/min/m^2 (lasting more than 1 hour) requiring low-dose inotropes
	Moderate PGD-LV: Must meet one criterion from I *and* another criterion from II:	I. *One* criteria from the following: Left ventricular ejection fraction $\leq$ 40%, *or* Hemodynamic compromise with RAP $>$ 15 mm Hg, PCWP $>$ 20 mm Hg, CI $<$ 2.0 L/min/m^2, hypotension with MAP $<$ 70 mm Hg (lasting more than 1 hour) II. *One* criteria from the following: i. High-dose inotropes—Inotrope score $>$ 10[a] *or* ii. Newly placed IABP (regardless of inotropes)
	Severe PGD–LV	Dependence on left or biventricular mechanical support including ECMO, LVAD, BiVAD, or percutaneous LVAD. Excludes requirement for IABP.
2. PGD-right ventricle (PGD-RV):	Diagnosis requires either both i and ii, or iii alone:	i. Hemodynamics with RAP $>$ 15 mm Hg, PCWP $<$ 15 mm Hg, CI $<$ 2.0 L/min/m^2 ii. TPG $<$15 mm Hg and/or pulmonary artery systolic pressure $<$ 50 mm Hg, *or* iii. Need for RVAD

BiVAD, biventricular assist device; CI, cardiac index; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; LVAD, left ventricular assist device; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; RVAD, right ventricular assist device; TPG, transpulmonary pressure gradient.

[a]Inotrope score = dopamine ($\times$1) + dobutamine ($\times$1) + amrinone ($\times$1) + milrinone ($\times$15) + epinephrine ($\times$100) + norepinephrine ($\times$100)[67] with each drug dosed in µg/kg/min.

Refers to text above: defenition and severity scale for primary graft dysfunction (PGD) from the 2013 ISHLT consensus conference

PGD is associated with poorer short (30 day) and long (1 and 5 year) term survival and with an increased need for re-transplantation.[95] Prolonged ischemic time is a risk factor for PGD and the subsequent development of CAV[96-98], and correlates with increased risk and frequency of rejection.[99] These complications have economic consequences; studies actively demonstrate that a decrease in ischemic time would reduce costs through reduction of length of stay in ICU (patients with PGD have longer length of stay in ICU).[100] Additionally, re-transplantation is frequently the only option for patients with severe PGD, or later on, CAV; this incurs further costs.

The mechanism of thymoglobulin in abrogating ischemia-reperfusion injury has been thought to result primarily from a direct effect on blocking the cell-to-cell interactions[69] and reducing the degree of leukocyte rolling and adhering along capillary endothelial surfaces.[101] This effect is due to down modulation of adhesion molecules and specific receptors which are responsible for these interactions (LFA-1, VLA-4, CCR5, and CCR7).[102] Thymoglobulin can also indirectly reduce inflammatory mediators and inhibit leukocyte-chemotaxis or chemokine receptor expression.[69,101,103] For that reason, inhibition of leukocyte homing and trafficking to the graft by binding to chemokine receptors is another way by which thymoglobulin affects IRI.[104] Additionally, TG reduces the number of peripheral lymphocytes from the circulating pool by inducing T-cell depletion through complement-related lysis or activation associated apoptosis.[105] Moreover, it causes anergy and functional impairment of non-depleted lymphocytes and prevents migration of memory T-cells.[102,105] Lopez *et al*. showed that the therapeutic effect of TG is not only due to T-cell depletion, but also due to generation of regulatory T-cell.[52] As a polyclonal agent, directed against molecules participating in IRI, it can minimize the IRI related problems in the grafted organ and subsequently preventing graft failure.[106,107]

Some experimental studies have been published to show benefits of ATG in reducing IRI. Preville *et al*. performed an experimental study in a non-human primate model to investigate the extent of T-cell depletion in lymphoid tissue after ATG usage. The purpose of this study was to establish a better concept of the mechanisms of action of ATG and to determine the appropriate dosage of ATG in different applications. Using skin grafts and heart transplantation models, ATG treatment induced a dose-dependent lymphocytopenia and T-cell depletion in spleen and lymph nodes due to T-cell apoptosis.[105]

Beiras-Fernandez *et al*. performed another study on two different groups of primates (Cynomolgus monkeys); one group was treated with ATG and the other one without ATG. The study was designed to evaluate the effect of ATG on the prevention of apoptosis in reperfused limb after ischemia and to monitor its ability to increase lymphocyte apoptosis. There was a significant decrease of apoptotic cells in skeletal muscle, connective tissue, and endothelial cells in the ATG treated animals after 60 minutes of warm ischemia. Additionally, white blood cell (WBC) infiltration in muscles was reduced while the apoptosis of WBCs was increased. Furthermore, mononuclear cells in peripheral blood, expression of adhesion molecules, and tissue damage were significantly decreased in the ATG treated animals. The authors concluded that ATG not only increased the rate of apoptosis in WBCs, but also protected the reperfused tissue against IRI.[69]

Together the data indicate that IR-initiated inflammation contributes to poorer short-term outcomes, risk/frequency of rejection, and development of late cardiac allograft vasculopathy, and that ATG may be able to improve this through reduction of IR injury.

1.2 DRUG BACKGROUND

1.2.1 Description and Mechanism of Action

Polyclonal antibodies were introduced in the late 1960s for the induction phase of immunosuppression in heart transplantation.[50] Historically, many different preparations were produced at, and used exclusively by, individual transplant centers, including anti-thymocyte, anti-lymphoblast, and anti-lymphocyte antibodies raised in rabbits, horses, and goats. **Over the past 40 years, rabbit antithymocyte globulin (ATG) has emerged as the most clinically efficacious of the polyclonal antibodies**[108]. ATG comprises a heterogeneous group of gammaglobulins (anti-CD2, CD3, CD4, CD8, CD18, HLA class I antigens and HLA-DR) that are produced by injecting human lymphocytes (or lymphocyte membranes) into a rabbit.[109] The rabbit then develops an immune response, which leads to the formation of gammaglobulins that are purified for pharmaceutical use in humans. **The mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. ATG is a pan-T-cell agent that when administered reduces lymphocyte counts to about 10% of normal values in humans.** The lymphocytopenia is attributed to several mechanisms, including complement dependent cytolysis, cell-mediated antibody-mediated cytolysis, as well as opsonization and subsequent phagocytosis by macrophages of the reticuloendothelial system.

"Induction immunosuppressive therapy" refers to the use of antithymocyte antibodies at the time of transplantation. **It is hypothesized that ATG induction therapy may enhance graft tolerance by blocking T-cell activation and other immune cell functions against the allograft at the time**

of transplantation. It has been shown that the probability of developing a high-grade rejection is markedly decreased six months after heart transplantation, resulting in the reduction of immunosuppression over time.[110] In addition, some clinical data seem to suggest that aggressive early rejection prophylaxis may alter the predisposition to reject and tolerate long after the period of early prophylaxis.[110,111] It is believed that this graft tolerance develops as a result of the gradual emergence of suppressor mechanisms through the selective clonal deletion of alloreactive T-cells. *In vitro* experiments have shown that ATG has (1) the highest ability to induce the apoptosis and activation-induced cell death of CD4+ T-cells when compared with anti-IL2R antibodies; and (2) a dose dependent ability to inhibit the proliferation of resting T-cells that had been activated by anti-CD3 mouse antibody.[112] It is thought that that this may be the mechanism by which ATG enhances graft tolerance.

Thymoglobulin® (Genzyme) [rabbit anti-thymocyte globulin (ATG)] is a purified pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains polyclonal cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. Thymoglobulin® (Genzyme) includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and β2-microglobulin (see Appendix F). *In vitro*, Thymoglobulin® (Genzyme) (concentrations >0.1 mg/mL) mediates T-cell suppression effects via inhibition of proliferative responses to several mitogens.[113-115] In patients, T-cell depletion is usually observed within a day from initiating Thymoglobulin® (Genzyme) therapy.[116-118]

Genzyme currently holds the license for Thymoglobulin® after acquiring SangStat in 2003. SangStat acquired an exclusive license in 1993 from Pasteur Merieux Connaught (PMC), a subsidiary of Rhone Poulenc S.A., to market this drug in the United States and Canada. Thymoglobulin or Thymoglobulin(e)-Merieux is manufactured and marketed outside North America by IMTIX, the Pasteur Merieux Connaught transplantation division, and was used in North American studies prior to marketing clearance from the FDA, which it received on December 30, 1998. Thymoglobulin and Thymoglobulin(e)-Merieux are the trade names still currently used outside the United States. Thus, to clarify, **Thymoglobulin® (Genzyme), Thymoglobulin® (Sangstat), Thymoglobulin, and Thymoglobulin(e)-Merieux are all trade names used for ATG produced using exactly the same protocol.**

1.2.2 Pharmacokinetics/pharmacodynamics

After an intravenous dose of 1.25 to 1.5 mg/kg/day (over 4 hours for 7–11 days) 4–8 hours post-infusion, Thymoglobulin® levels were on average 21.5 µg/mL (10–40 µg/mL) with a half-life of 2–3 days after the first dose, and 87 µg/mL (23–170 µg/mL) after the last dose. A group of 79 renal transplant patients were treated with Thymoglobulin®, 1.5 mg/kg/day for 6-14 days as part of a double-blinded trial comparing the efficacy of Thymoglobulin® and Atgam (horse anti-thymocyte globulin) for acute rejection[109]. Serial serum samples from the patients were tested to determine the level of Thymoglobulin® (i.e. rabbit IgG levels = total Thymoglobulin®) and anti-Thymoglobulin using ELISAs. Antibodies binding to human lymphocytes (active Thymoglobulin), were determined by flow cytometry; **no correlation was seen between treatment efficacy and either active or total Thymoglobulin concentrations; the overall treatment success rate was 86%.** Pharmacokinetics of total and active Thymoglobulin® were distinctly different; active Thymoglobulin® disappeared much more rapidly: only 12% of patients had detectable active Thymoglobulin® by day 90 compared to 81% of patients with detectable total Thymoglobulin®

percent active Thymoglobulin® decreased from a peak of 0.56-0.7% during treatment, to 0.07-0.35% by day 21, and less than 0.14% by day 30. Thymoglobulin® and active Thymoglobulin® concentrations were modeled by multiple regression. Using dose number and sensitization as independent variables, 47-76% of the variability seen in interpatient Thymoglobulin® levels could be explained, while for active Thymoglobulin® levels, the measured variables accounted for 13-48% of the observed interpatient variation.

The authors concluded that: (1) for a group of patients receiving primary Thymoglobulin® treatment (averaging nine full and one partial dose per patient), **neither Thymoglobulin® nor active Thymoglobulin® levels are predictive of treatment outcome;** (2) active Thymoglobulin® disappears more rapidly from the circulation than total Thymoglobulin®; and (3) patients that develop anti-rabbit IgG antibodies clear Thymoglobulin® and active Thymoglobulin® more rapidly than unsensitized patients.[119] However, another study found that after multiple administrations, Thymoglobulin® clearance decreases, resulting in an extended half-life. Thymoglobulin® can be detected in the serum of recipients up to 50 days after the last dose.[109] There are a few case reports of Thymoglobulin® being detected in the serum for up to a year after the last dose.

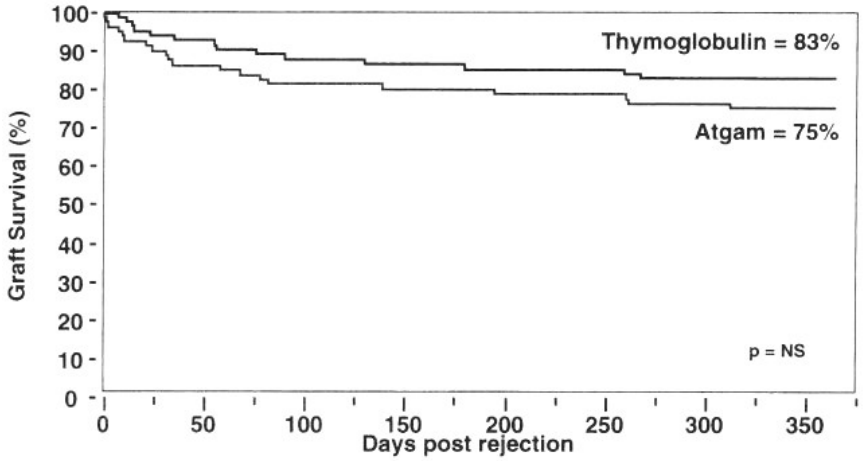
Thymoglobulin® administration is associated with significant T-cell subset depletion, general leucopenia, anemia, and thrombocytopenia that recovers upon cessation of therapy (see graphs below). However, persistent, mild leucopenia lasting up to a year after Thymoglobulin® has been observed in some case reports, though the leucopenia could have also been attributed to other immunosuppressive agents, such as cyclosporine, mycophenolate mofetil, tacrolimus, etc.

1.2.3 Clinical Trials

Most of the randomized human clinical trials using ATG are in the area of renal transplantation. **ATG has been demonstrated to be efficacious and safe for use in acute rejection in renal (adult and pediatric) transplantation,[109,120] intra-operative induction therapy in renal transplantation,[106,121] induction therapy in pediatric cardiac transplantation,[47] as well as outpatient infusion for acute renal rejection[122].**

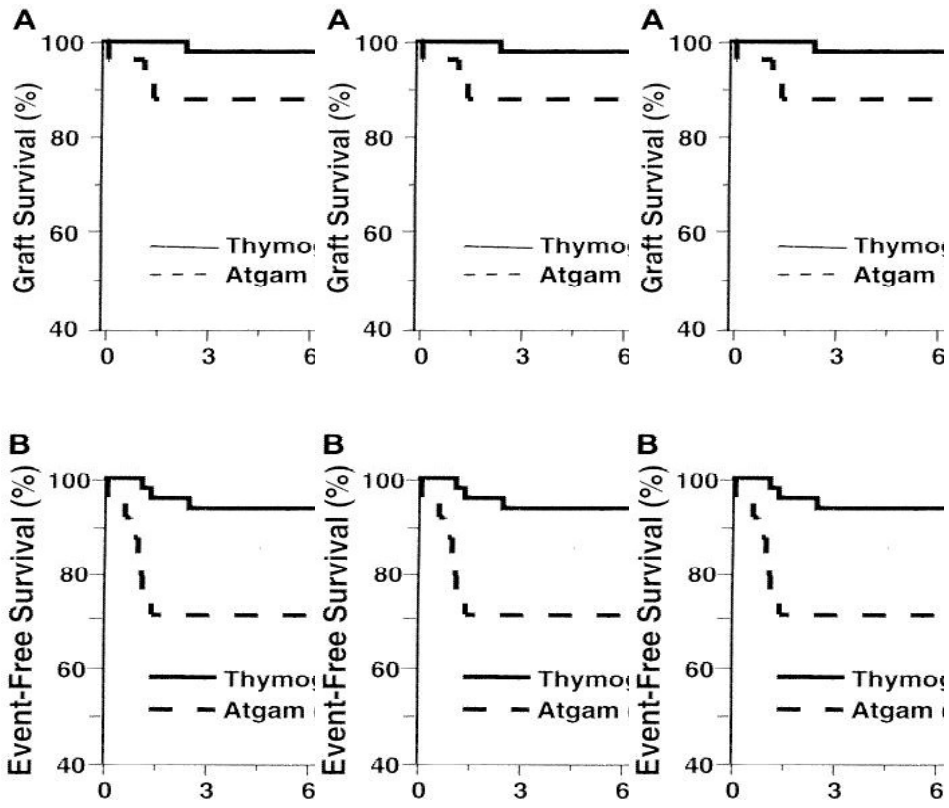
1.2.3.1 Human Clinical Trials of ATG in Renal Transplantation

(a) The marketing clearance application for Thymoglobulin® was based on a single double-blinded, randomized, multi-center Phase III trial, which as conducted at 28 leading U.S. renal transplant centers. In this trial, **Thymoglobulin® was compared to Atgam (horse-derived polyclonal antibody) in the treatment of renal transplant patients (n=163) with biopsy-proven Banff grade 2 (moderate), grade 3 (severe), or steroid-resistant grade 1 (mild) acute graft rejection.** Patients were randomized to receive 7 to 14 days of Thymoglobulin® (1.5 mg/kg/day) or Atgam (15 mg/kg/day). For the entire study, the two treatment groups were comparable with respect to donor and recipient characteristics. **There were no significant differences between the two treatments with respect to (i) day 30 serum creatinine levels relative to baseline; (ii) improvement rate in post-treatment histology; (iii) one year post rejection Kaplan-Meier patient survival (Thymoglobulin® 93%, n=82 and Atgam 96%, n=80); (iv) day 30 and (v) one-year post rejection graft survival (Thymoglobulin® 83%, n=82 and Atgam 75%, n=80).**



Refer to section (a) above: Overall 1-year post-rejection therapy actuarial graft survival (Kaplan-Meier method), by acute rejection severity for renal transplant recipients treated with either Thymoglobulin or Atgam.

(b) **In another study also comparing the efficacy and safety of Thymoglobulin® to Atgam for induction in adult renal transplant recipients**, 72 patients were randomized 2:1 in a double-blinded fashion to receive Thymoglobulin® (n=72) at 1.5 mg/kg/day intravenously or Atgam (n=24) at 15 mg/kg/day intravenously, intraoperatively, then daily for at least 6 days. By 1 year after transplantation, 4% of Thymoglobulin® treated patients experienced acute rejection compared with 25% of Atgam treated patient (p=0.14). Both the rate and severity of acute rejection was significantly lower in with Thymoglobulin® than Atgam and no recurrent rejection occurred with Thymoglobulin® compared with 33% with Atgam (but p=NS). Patient survival was not different, but **the composite end point of freedom from death, graft loss, or rejection, the "event-free survival," was superior with Thymoglobulin® (94%) compared with Atgam (63%, p=0.0005). Fewer adverse events occurred with Thymoglobulin® (p=0.013).** Leucopenia was more common with Thymoglobulin® than Atgam (56% vs 4%, p<0.0001) during induction. The incidence of cytomegalovirus disease was less with Thymoglobulin® than Atgam at 6 months (10% vs 33%, p=0.025).[123]



Refer to section (b) above: Graph A: Kaplan-Meier allograft survival. Allograft survival was superior with Thymoglobulin® compared with Atgam, *P*=0.021, log-rank test when graft losses from all causes were considered. Numbers in parentheses are the number of patients remaining at risk. Graph B: Kaplan-Meier event-free survival. Event-free survival, defined as freedom from rejection, death, and allograft loss, was superior with Thymoglobulin® compared with Atgam. *P*=0.0005, log-rank test. Numbers in parentheses are the number of patients remaining at risk.

(c) **An open, randomized, multicenter trial investigated induction therapy in non-hyperimmunized patients receiving their first cadaveric renal allograft with either Thymoglobulin® (1.0-1.5 mg/kg/day [dose adjusted daily to keep CD2 or CD3 counts below 20/mm^3] plus delayed cyclosporine (n=50) or basiliximab [an inhibiting IL-2 receptor monoclonal antibody] (20mg/day on days 0-4) plus early cyclosporine (n=50).** All the patients were also treated with steroids and mycophenolate mofetil. **Patient and graft survival rates at 12 months were 98 and 94% in the basiliximab group, respectively, compared with 100 and 96% in the Thymoglobulin® group.** The incidences of biopsy-confirmed acute rejection (8% in both) and treatment failure (8% in Thymoglobulin® and 14% in basiliximab) were not significantly different. The incidence of CMV infection and need for dialysis was also not statistically significantly different between the two groups.[124]

Refer to text in section (c) above:

Table 3: Incidence of efficacy variables during the 6-month post-transplantation period (ITT population)[a]

Variable	Basiliximab (n = 50)	Thymoglobulin (n = 50)
First biopsy-confirmed rejection episode 4	(8.0%)	4 (8%)
First acute rejection episode treated with antibody therapy	0	1 (2%)
First acute rejection episode treated with tacrolimus	1 (2.0%)	0
Graft loss[b]	2 (4.0%)	0
Death	1 (2.0%)	0
Treatment failure[c]	7 (14.0%)	4 (8%)

[a]The differences between the treatment groups are not statistically significant.
[b]Graft loss was defined as the need for regular postoperative dialysis, graftectomy, or retransplantation.
[c]Treatment failure was defined as acute rejection, graft loss, or death, whichever occurred first.

(d) **Thymoglobulin® induction has been shown to be safe and effective in combination with triple immunosuppressive therapy for preventing early rejection in pediatric renal transplant recipients.** Seventeen pediatric renal transplant recipients (mean age 10.1+/-5.2 years) received either primary or second transplants between 1 August 1999 and 31 July 2001. One patient had primary allograft non-function secondary to vascular thrombosis. Two patients (12%) had delayed allograft function. Immunosuppression consisted of Thymoglobulin® induction (1.5 mg/kg/dose for a mean number of doses 6+/-1.7) with tacrolimus (62%) or cyclosporine A (38%), mycophenolate mofetil, and prednisone. **One year post transplant, patient and graft survival was 100% and 93%, respectively. No acute rejection episodes occurred during the first 6 months after transplantation in any of the recipients.** Additionally, no rejection episode occurred among the 14 patients followed for 1 year after transplant. The incidences of asymptomatic cytomegalovirus (CMV) and Epstein-Barr virus (EBV) seroconversion at 1 year in seronegative recipients with a seropositive donor were 100% of 4 patients and 0% of 4 patients, respectively. **No symptomatic CMV or EBV infections and no post-transplant lymphoproliferative disease occurred in any patient over the course of 1 year of follow-up.**[125]

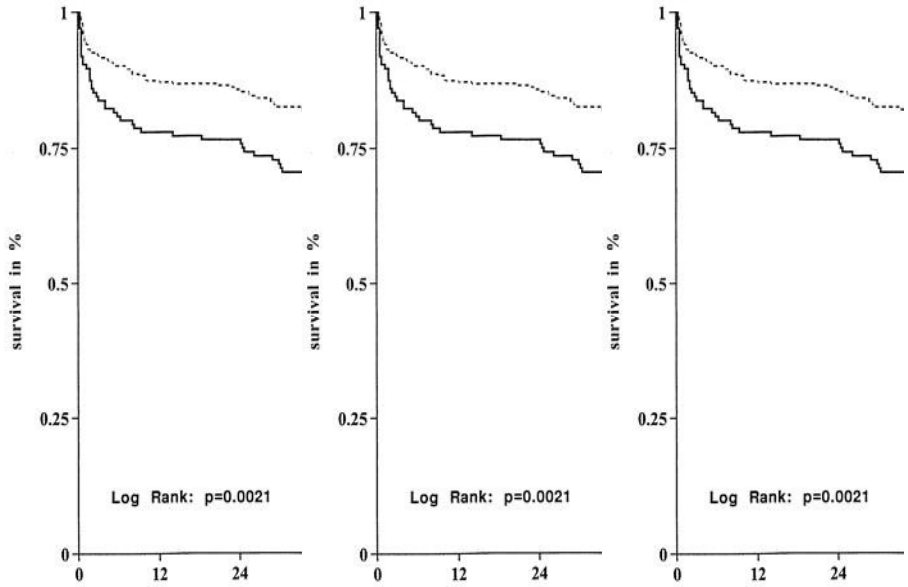
Acute rejection episodes	
6 months (16 patients)	0
12 months (14 patients)	0
Estimated creatinine clearance	
Baseline (ml/min per 1.73 m^2)	77.3±23.7
3 months	77.9±24.2
6 months	88.2±28.2
12 months	74.2±14.8

Refer to text in section (d) above: Short-term outcome for 16 pediatric kidney transplant recipients who received Thymoglobulin® induction therapy

1.2.3.2 Human Clinical Studies of ATG in Cardiac Transplantation

To date all studies of ATG in cardiac transplantation have been either uncontrolled, retrospective, or comparing another agent whose efficacy is unknown.

(a) **A total of 484 primary cardiac transplanted patients received induction therapy with two different rabbit-ATG (Thymoglobuline-Merieux: n=342, ATG-Fresenius: n=142).** All patients received immunosuppressive maintenance therapy with cyclosporine, azathioprine, and prednisolone. Cardiac rejection was assessed by serial endomyocardial biopsies. Surveillance of graft arteriosclerosis was performed by angiograms 1, 3, and 5 years after transplantation. **Five-year survival was significantly better in the Thymoglobuline group (76 vs. 60%). Thymoglobuline-Merieux patients had a lower rate of death from rejection (2.3 vs. 10%; P<0.01) and graft arteriosclerosis (0.88 vs. 5.6%; P<0.01).** After 5 years, freedom from rejection was 72% in the Thymoglobuline-Merieux group compared to 42% in the ATG-Fresenius group (P<0.01). Graft arteriosclerosis appeared in 14% of Thymoglobuline patients and in 28% of ATG-Fresenius patients (P<0.01). Viral infections occurred more often in Thymoglobuline patients (53 vs. 39%, P<0.05) although there was no difference in appearance of cytomegalovirus disease (17 vs. 13%). Freedom from posttransplant malignant disease was comparable between the two groups[67].

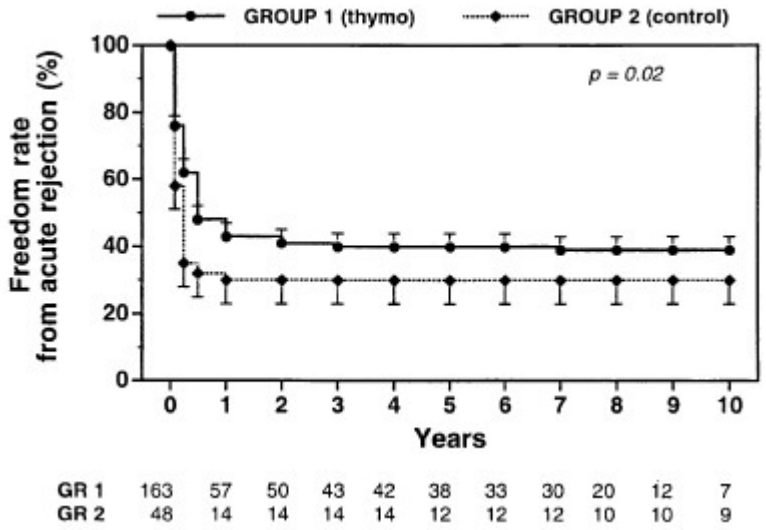


TA	TA	TA
Rejection	Rejection	Rejection
Infection	Infection	Infection
Graft arterioscleros	Graft arterioscleros	Graft arterioscleros
Lymphoid cancer	Lymphoid cancer	Lymphoid cancer
Nonlymphoid cance	Nonlymphoid cance	Nonlymphoid cance
Other	Other	Other
Total deaths	Total deaths	Total deaths

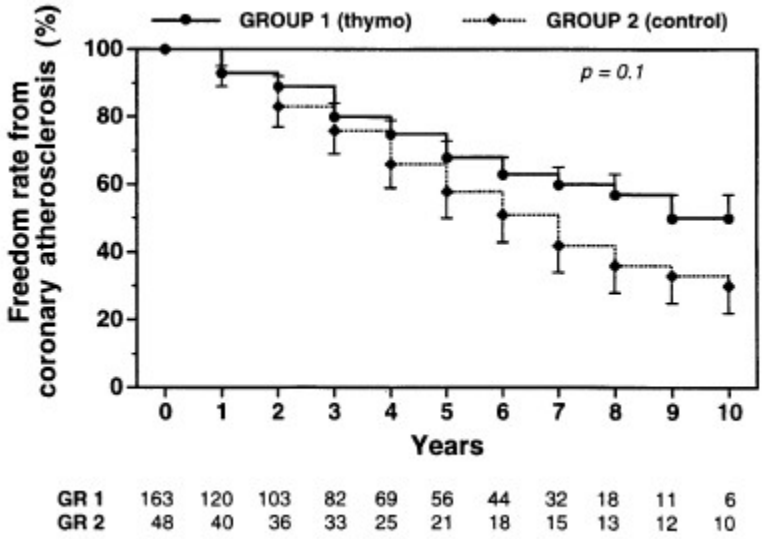
Refer to text of section (a) above: Actuarial survival and causes of death up to 10 years after cardiac transplantation comparing thymoglobuline versus ATG-fresenius

(b) **A retrospective analysis of 163 patients who were administered a 3-day course of intravenous Thymoglobuline-Merieux immediately following heart transplantation from 1988 to 1998 (called Group 1) compared with 48 patients transplanted from 1983 to 1987 and during an isolated period in 1994 where no induction therapy was used and intravenous and oral cyclosporine was used immediately following heart transplantation (called Group 2).** Routine endomyocardial biopsies were performed in all patients. One, 5- and 10-year actuarial survival rate averaged 85%+/-3, 77%+/-4 and 67%+/-5 in Group 1 compared with 88%+/-5, 81%+/-6 and 76%+/-6 in Group 2 (p = 0.5). At 1 year, **the freedom rate from an episode of acute rejection averaged 43%+/-4 in Group 1 and 30%+/-7 in Group 2 (p = 0.03)** and the freedom rate from an episode of infection averaged 44%+/-4 in Group 1 and 31%+/-7 in Group 2 (p = 0.2). At 1, 5 and 10 years, the freedom rate from graft coronary artery disease averaged 93%+/-2, 68%+/-5 and 50%+/-7 in Group 1 compared with 93%+/-4, 58%+/-8 and 30%+/-8 in Group 2 (p = 0.1) and the freedom rate from cancer averaged 98%+/-1, 91%+/-3 and 67%+/-8 in Group 1 compared with

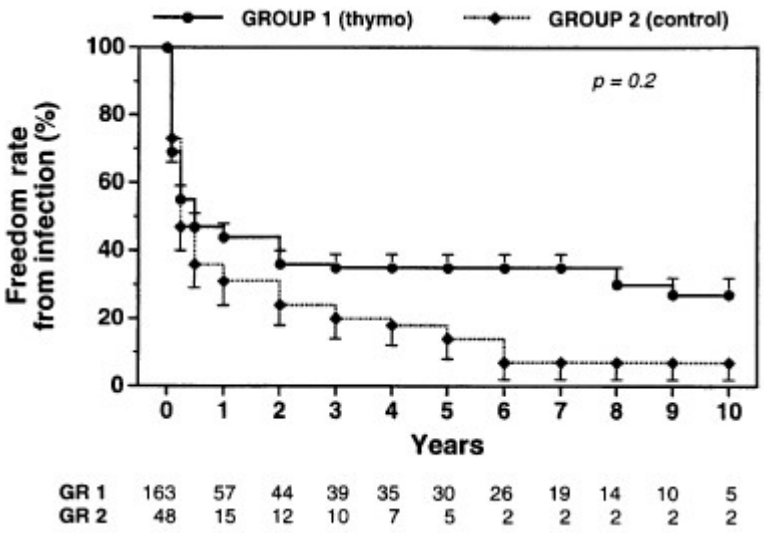
100%, 95%+/-3 and 77%+/-8 in Group 2 (p = 0.2). There was no side effect related to the systemic injection of Thymoglobuline-Merieux. Thus, **after induction with Thymoglobuline-Merieux, the risk of infection and malignancy was not increased** and there was a non-significant trend towards a lower incidence of coronary atherosclerosis 5 and 10 years after transplantation.[42]



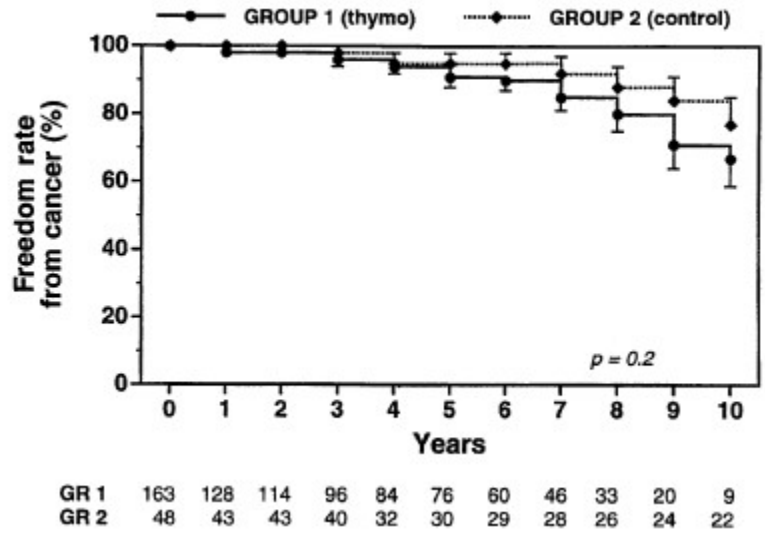
Refers to text in Section (b): Actuarial freedom rate from rejection. Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control)



Refers to text in Section (b): Actuarial freedom rate from CAV.
Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).



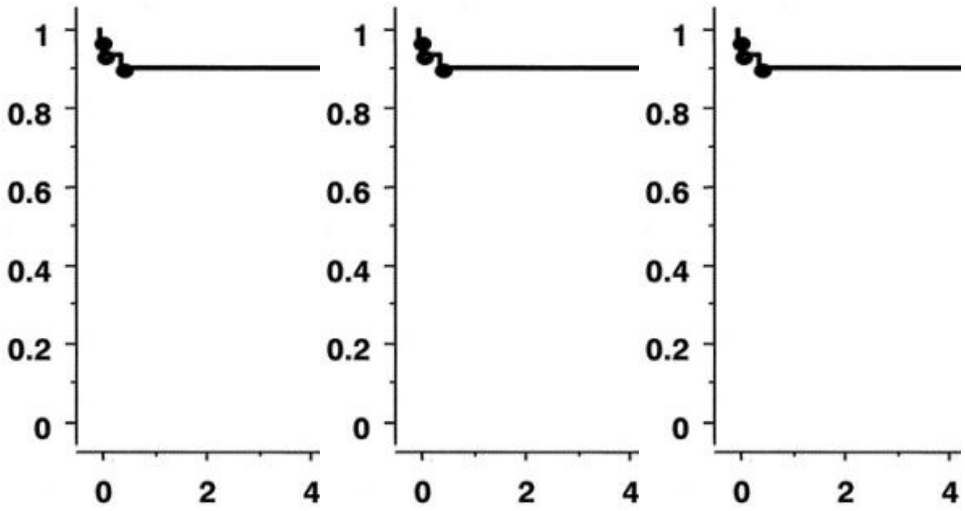
Refers to text in Section (b): Actuarial freedom rate from infection. Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).



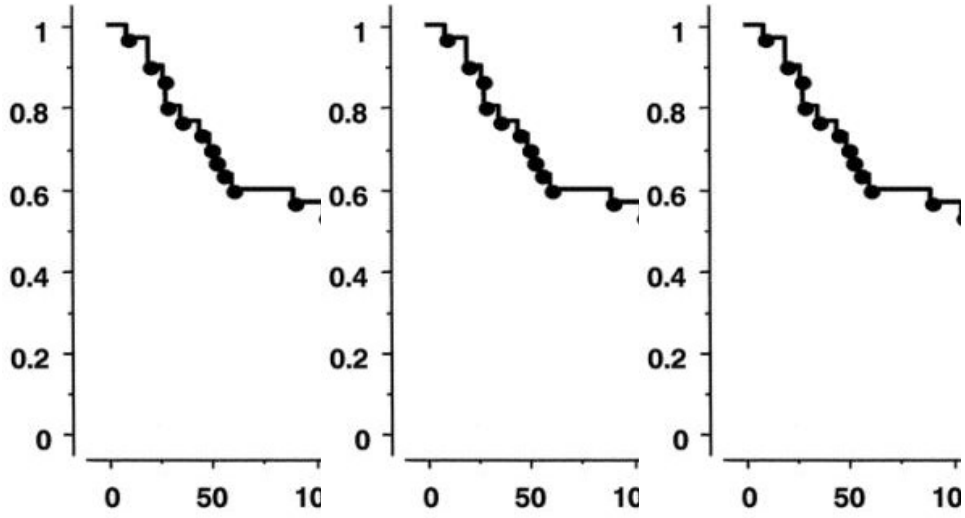
Refers to text in Section (b): Actuarial freedom rate from cancer.
Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).

(c) Another **retrospective study examined the use of Thymoglobuline-Merieux in pediatric cardiac transplantation over a 13-year period in a single-center** with respect to the short-term hematological effects as well as longer-term outcomes. The dose of Thymoglobuline given depended on baseline platelet count and was 2, 1.5, or 1 mg/kg per day over 5 days for the following platelet count groups: greater than 150,000/mm (normal group), 100 to 150,000/mm (mild thrombocytopenia group), and 50 to 100,000/mm (moderate thrombocytopenia group). Thirty children of median age 14.2 years were given a median cumulative dose of Thymoglobuline of 8 mg/kg per patient; the moderate thrombocytopenia subgroup was given significantly less (6.4 mg/kg) (P=0.032). Immediate tolerability of Thymoglobuline was good, with no cases of first-dose

syndrome, anaphylaxis, or serum sickness. The platelet count decreased at the start of therapy, but recovered after discontinuation, and did not give rise to clinical concern. Patients were followed up for a median of 6.3 years (7 days-15.5 years); actuarial survival was 90%, 86%, and 74.5%, respectively, at 1, 5, and 10 years. In the first year, 50% of patients suffered an episode of rejection. The overall incidence of infection in the month following transplantation was 40%. One lymphoma occurred at 5 months**. The authors concluded that the use of Thymoglobuline-Merieux in pediatric heart-transplant patients as part of an immunosuppressive protocol, with dose adjustment according to platelet levels, is effective in terms of a decreased rejection rate and improved patient survival, and safe in terms of the incidence of infections and malignancy.**[21]



Refer to text in section (c): Patients' actuarial survival rates at 1, 5, and 10 years (n=30).



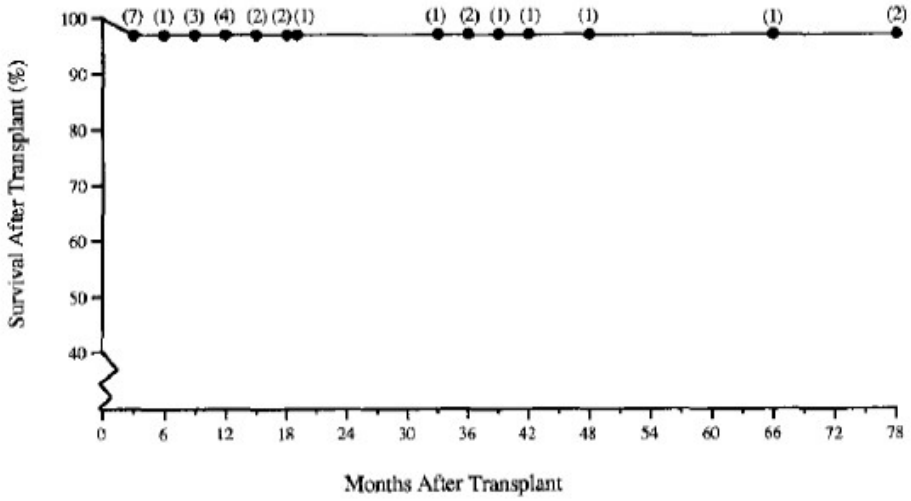
Refer to text in section (c): Freedom from cytomegalovirus with time (n=30).

(d) In still yet another single-center, retrospective study**, 31 consecutive heart transplant recipients (mean age, 7.8 years; median age, 9 years; range, 4 months-17 years), who all**

survived surgery had induction with Thymoglobuline-Merieux at age-dependent doses (1-1.5 mg/kg/day between 0 and 1 year; 1.5-2 mg/kg/day from 1 year to 8 years; and 2.5 mg/kg/day >8 years). Duration of treatment was 1 to 7 days. In patients <1 year old, the total number of lymphocytes was maintained at >500/mm^3, in patients between 1 and 8 years old >300/mm^3, and in patients >8 years old >150/mm^3. **Thirty of 31 patients were alive at the end of follow-up.** During the first 3 months, 3 Grade 3A and 10 Grade 1A (Working Formulation grading system) rejection episodes occurred. All reversed after steroid treatment. Eleven viral infections, 2 bacterial infections, and 1 fungal infection occurred. Not all patients with infection were symptomatic but all responded successfully to treatment. **Given that there was no comparison group, it is difficult to infer a possible increase in the rate of infection, but in the discussion the authors remarked that their rate of infection did not "substantially differ from risks reported in the literature for patients who did not receive induction therapy."** One episode of post-transplantation lymphoproliferative disease regressed after decreasing immunosuppression therapy and after acyclovir therapy**. The authors concluded that immunosuppression therapy with Thymoglobuline is safe even in early infancy.**[126]

(e) In another **retrospective, single-center study 30 patients (ages 8 months to 24 years) with end-stage heart failure underwent cardiac transplantation:** 12 (40%) for postoperative end-stage heart failure, 9 (30%) as primary treatment for congenital heart disease, 5 (17%) for dilated cardiomyopathy, and 4 (13%) for restrictive/hypertrophic cardiomyopathy. Nineteen patients (63%) had undergone prior operations; 4 patients received transplants for failed Fontan procedures. Induction therapy with rabbit antithymocyte globulin (DCI Laboratories) was used routinely, and long-term immunosuppression was by cyclosporine and azathioprine alone. Rejection surveillance/diagnosis was based on echocardiographic criteria. Post transplantation follow-up ranges from 3 to 78 months. Operative mortality was 3.3% (1/30). **No patients were diagnosed with either infection, accelerated allograft atherosclerosis, or post transplantation lymphoproliferative disease.**[47]

(f) A retrospective, multicenter study in the United Kingdom was performed, encompassing over 2,000 patients between 1995 and 2008, 1000 of whom had been inducted with ATG. The study found no significant difference in survival at 10 years between the two groups; 56.2% in the ATG group versus 55.9% in the no-ATG group ($p = 0.95$). The investigators did note lower rates of rejection over the first year (incidence rate ratio, 0.76; 95% confidence interval [CI], 0.68–0.85, $p < 0.01$), but this potential benefit was accompanied by increased rates of infection.[127]



Refer to text in section (e): Survival after transplantation. There has been one operative death (3.3%) and no deaths in post-transplantation follow-up.

1.3 RATIONALE FOR PERFORMING THE STUDY

1.3.1 The Efficacy of ATG Induction in Cardiac Transplantation is Unknown

There is limited clinical research examining the efficacy of ATG at reducing the incidence of *de novo* donor specific antibody production after heart transplantation. A study in kidney transplantation demonstrated a lower incidence of *de novo* DSA in moderately sensitized patients. A series of 114 consecutive, moderately sensitized kidney transplant patients received either ATG (n = 85) or basiliximab (n = 29) induction. At 1 year post-transplant, patients who received ATG induction had a decreased risk of developing *de novo* DSA (HR 0.33, 95% CI 0.09-1.24) and the group as a whole had a lower sum total *de novo* DSA level as measured by MFI (455 versus 3,652 p = 0.02).[128] In a single-center, retrospective study of 217 consecutive sensitized (PRA ≥ 10%) heart transplant recipients, patients were divided into those receiving ATG (n = 162) and those who did not (n = 55). Patients treated with ATG had greater freedom from *de novo* DSA development compared to no ATG at 1 year after transplant (86.4% versus 74.5%, p = 0.038).[129]

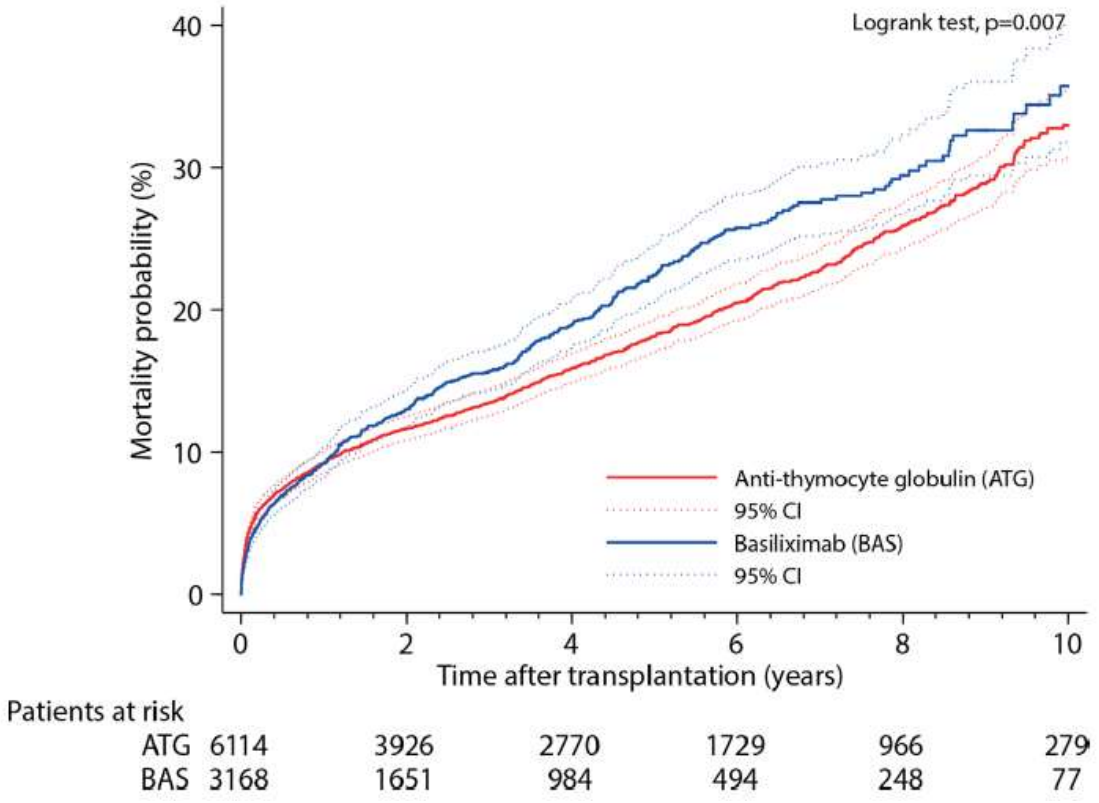
Ischemia-reperfusion injury (IRI) can damage the allograft vascular endothelium leading to vasculopathy. Animal models have demonstrated that ATG reduces IRI. A study of cynomolgus monkeys found that circulating leukocytes were lower in monkeys given ATG 30 minutes prior to reperfusion of ischemic limbs.[130] In a similar study, expression of adhesion molecules (ICAM-1, VCAM, PECAM, CD11b, CD62E) and inflammatory cytokines (IL-1, IL-6, TNF-α) were decreased in the ischemic limbs of cynomolgus monkeys treated with ATG 30 minutes prior to reperfusion.[131] A human model utilizing human umbilical vein endothelial cells (HUVECs) corroborated these findings. HUVECs were bathed in a solution of ATG before and after stimulation with TNF-α. Cells that were not bathed in ATG served as controls. Adhesion molecule expression (ICAM-1 and CD62E) was reduced in the ATG group.[132] A study of human serum demonstrated that ATG modulates leukocyte responses by affecting cellular adhesion molecules and chemokine receptors.[102] Studies in heart transplant recipients are limited to a single retrospective study. A series of 330 consecutive heart transplant patients was divided into those who received ATG

induction and those who did not. Patients who received ATG induction (n = 129) had significantly greater freedom from any ischemia-reperfusion injury on biopsy in the first month after transplant compared with patients receiving no ATG (n = 201) (74.8% vs 63.5%, p = 0.019).[133]

Clinical evidence for the use of peri-operative induction therapy to prevent CAV and rejection in adult cardiac transplantation is limited. Whether to use cytolytic therapy to either prevent rejection or treat acute rejection has been based on personal preferences because, to date, there have not been any randomized clinical trials conducted to evaluate the efficacy of ATG induction therapy in cardiac transplantation.

A small number of trials have examined the effects of induction therapy in cardiac transplantation. Two of these were randomized control trials looking at IL-2R antagonists versus no induction and showed a reduction in the risk of rejection but no survival benefit.[134,135] Two further trials compared ATG induction against IL-2R antagonist induction.

However, **the majority of the retrospective studies in heart transplant recipients do suggest that ATG induction may be beneficial.** A large retrospective study examined 9,324 heart transplant recipients in the ISHLT database who received induction therapy with either ATG or basiliximab. The study found that patients treated with basiliximab had higher mortality rates, higher rates of infection, and suffered more episodes of graft failure than patients treated with ATG.[136]



Refers to text in above paragraph: Comparison of all-cause mortality probability between the basiliximab and ATG groups

A recent retrospective study compared 196 heart transplant recipients at a single center who received ATG induction therapy for renal insufficiency with patients who received no induction therapy. ATG appeared to dampen the formation for *de novo* DSAs in the first 12 months post-transplant.[137]

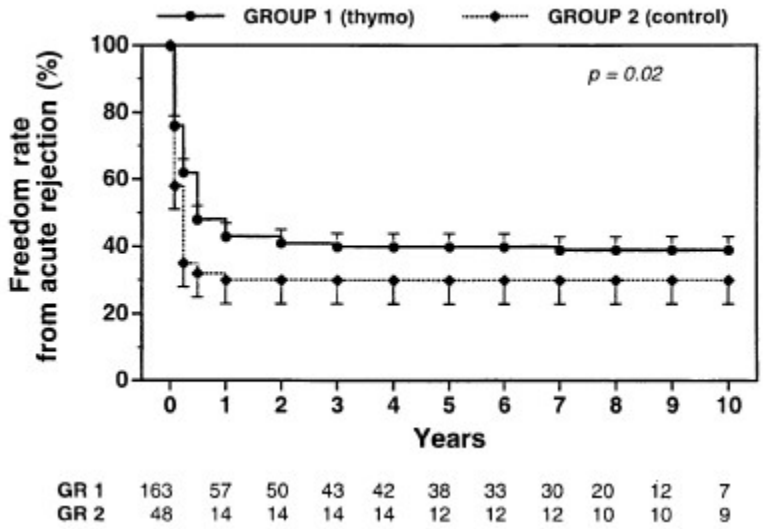
In another retrospective study 662 adult cardiac recipients who had received triple-drug immunosuppressive maintenance together with ATG induction therapy were evaluated for the presence and severity of CAV. ATG induction therapy was shown to have a protective effect against the development of CAV.[43,138]

Multivariate Analysis of Risk Factors Affecting Development of CAV After Cardiac Transplantation

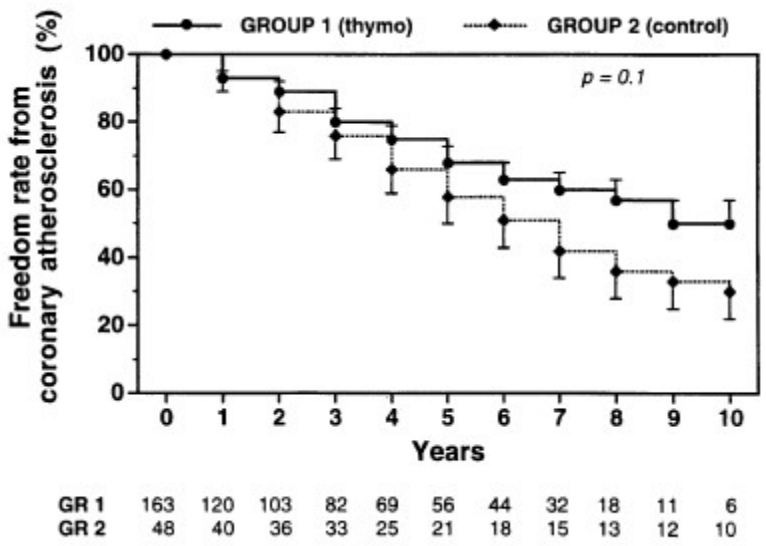
Risk factor	Risk factor	Risk factor	Risk factor	Risk factor
P	P	P	P	P
Thymoglobulin	Thymoglobulin	Thymoglobulin	Thymoglobulin	Thymoglobulin
Rejection >ISHLT grade 0	Rejection >ISHLT grade 0	Rejection >ISHLT grade 0	Rejection >ISHLT grade 0	Rejection >ISHLT grade 0
Male recipient	Male recipient	Male recipient	Male recipient	Male recipient
Older donor age	Older donor age	Older donor age	Older donor age	Older donor age
Higher cholesterol	Higher cholesterol	Higher cholesterol	Higher cholesterol	Higher cholesterol
Obesity posttransplant	Obesity posttransplant	Obesity posttransplant	Obesity posttransplant	Obesity posttransplant
IV antirejection therapy	IV antirejection therapy	IV antirejection therapy	IV antirejection therapy	IV antirejection therapy

Table refers to text in the paragraph above[43]

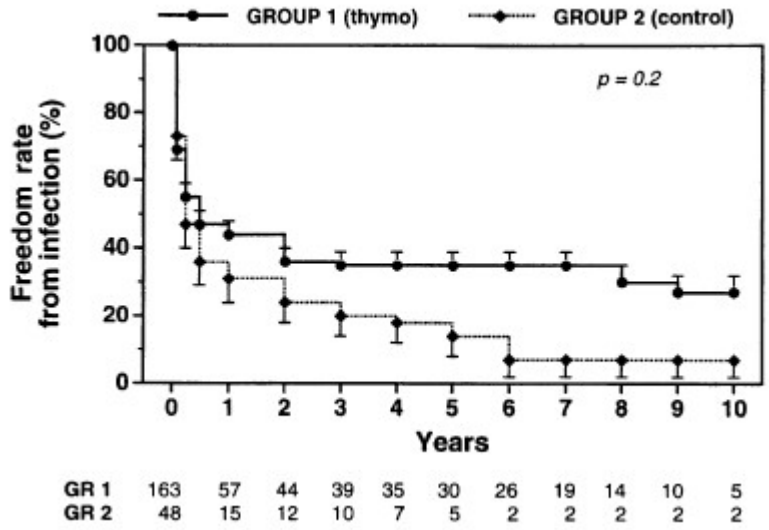
In another retrospective analysis, 163 adult patients transplanted between 1988 and 1998 who were administered a 3-day course of ATG induction were compared with a cohort of patients who underwent the same immunosuppressive therapy without ATG induction between 1983 and 1987. **ATG induction was associated with a significantly lower rate of acute rejection and a trend towards a decrease in the incidence of CAV. Moreover, the risk of infection and cancer was not increased.[42]** See next four figures:



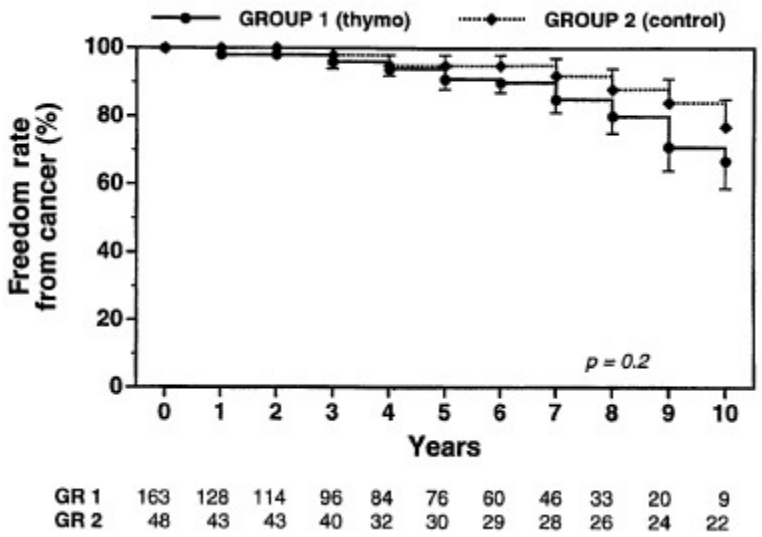
Refers to text in above paragraph: Actuarial freedom rate from rejection.
Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control)



Refers to text in above paragraph: Actuarial freedom rate from CAV. Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).



Refers to text in above paragraph: Actuarial freedom rate from infection. Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).

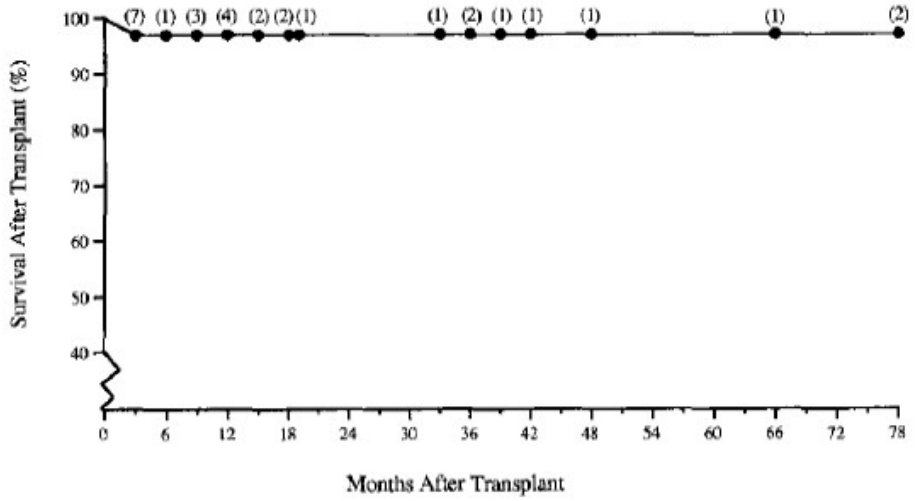


Refers to text in above paragraph: Actuarial freedom rate from cancer. Group 1=patients treated with thymoglobuline (Thymo). Group 2=patients not treated with thymoglobuline (Control).

Another retrospective analysis of 40 adult cardiac recipients treated with 5 days of ATG induction therapy did not show a difference in morbidity, but also confirmed the relative safety of ATG in that there was **no increase in the incidences of CAV, malignancy, or infection.**[139]

Results of another retrospective study in which 117 adult patients undergoing cardiac transplantation were administered a 3-day course of ATG induction also showed **a significantly lower early rejection rate**. Although patient survival was similar to results reported by others, **ATG induction was again not found to be associated with an increased incidence of malignancy or infection.**[140]

In a case series report 30 pediatric patients (age >6 months) who underwent cardiac transplantation between 1988 and 1994 at a center where ATG induction was used routinely were followed. **Over the eight years, there were no re-transplantations, no diagnoses of CAV, and no occurrence of post-transplantation lymphoproliferative disease.**[47]



Refer to text in paragraph above: Survival after transplantation. There has been one operative death (3.3%) and no deaths in post-transplantation follow-up.

The utility and applicability of the retrospective studies discussed above is limited by either the lack of controls or the use of recipients from nearly a decade earlier as controls for comparison. It is well documented that the clinical practice of heart transplantation has changed significantly from decade to decade with a constant improvement in survival and decrease in rejection rate.[55] Given this limitation one cannot attribute these outcomes purely to ATG induction therapy.

Whether ATG induction in adult heart recipients should be used cannot be based on studies of non-heart organ transplants or even the literature on pediatric heart transplantation because the immunogenicity of the cardiac allograft differs from that of other organ allografts and the pediatric immune system is markedly different from that of the adult.[141] Lastly, it is well known that *in vitro* studies do not necessarily always translate into the predicted clinical outcome. Therefore, whether or not perioperative ATG induction therapy is clinically beneficial with respect to decreasing rejection and preventing CAV remains theoretical.

1.3.2 Thymoglobulin has numerous mechanisms of immunosuppression

Some retrospective studies have suggested that aggressive early rejection prophylaxis using ATG may alter the predisposition to reject and tolerate the graft.[21] These beneficial effects were seen long after the period of early prophylaxis. These studies also correlated with other retrospective studies of ATG treated recipients which demonstrated long term improvement in survival and other end points such as rejection and CAV.[43,138] As mentioned above in the "Disease Background" (Section 1.1.1), CAV is thought to be associated with AMR and cellular rejection and related to both the time to onset of rejection as well as frequency of rejections. Thus it is reasonable to hypothesize that ATG may inhibit CAV by preventing rejection. As discussed in Section 1.2.1, ATG may enhance graft tolerance (and thereby prevent acute rejection) by inducing the apoptosis and activation-induced cell death of CD4+ T-cells, inhibiting IL-2 receptor expression, and inhibiting the proliferation of activated T-cells.[112,114] Another possible mechanism is through preventing the formation of anti-donor antibodies as ATGs have been found to inhibit B cell proliferation and differentiation into immunoglobulin-secreting cells.[113] Another possible mechanism is through inhibiting the formation of immune complex deposits by directly or indirectly inactivating the

various components of these deposits (i.e. complement, immunoglobulins, and fibrinogen [see Section 1.1.2]) and thus preventing the vascular injury of AMR. Although ATG is believed to be a heterogeneous group of predominantly anti-T-cell gammaglobulins (anti-CD2, CD3, CD4, CD8, CD18), antibodies directed against HLA class I antigens, HLA-DR, and beta2 microglobulin are inexplicably present. Thus, strangely the activity of ATG appears to expand beyond the realm of merely modulating T-cell activity. From this one could possibly hypothesize that the direct binding of ATG to the pre-/peri-operatively injured endothelium may shield the exposed donor antigens from the recipient's immune system, thus pre-emptively arresting the initial events involved in development of CAV.

Human and animal studies have demonstrated the ability of ATG to selectively delete activated T-cells and activated B-cells, to inhibit cytokine release by dendritic cells and activated T-cells, and to modulate adhesion and cell-trafficking molecules, thereby suppressing the activation of key early host immune responses to the graft.[50] In addition to these mechanisms there is evidence that ATG can also promote the phenotype and expansion of regulatory T-cells and immature DC. Two recent studies have demonstrated that *in vitro* treatment of lymphocytes with ATG caused the selective expansion of T-regs by promoting the conversion of CD4+CD25- cells to CD4+CD25+ cells followed by rapid proliferation.[52] These ATG-generated T-reg are capable of suppressing the immune responses of activated lymphocytes *in vitro.*[52,53] Futhermore adoptive transfer of ATG-treated lymphocytes into animal models of graft versus host disease caused increased survival and this effect appeared to be from the inhibition of allogeneic CD8+ T-cell expansion *in vivo.*[52] ATG also promotes the expansion of the natural killer regulatory T-cells (NK-T). Mice pre-treated with total lymphoid irradiation (TLI) and ATG have significantly improved survival after bone marrow transplant. The effect is attributed to NK-T-cells because the effect of TLI/ATG pre-treatment is absent in mice that are NK-T deficient.[50]

1.4 SIGNIFICANCE OF STUDY

Despite major advances in medical therapy, CAV remains the major factor limiting long term survival in heart transplant recipients. The limited options for these patients justify the need to seek potential alternative treatment options.

The majority of the retrospective studies in both renal and heart transplant recipients suggest that Thymoglobulin® induction has a protective effect against the development of graft vasculopathy.[42-44] Transplant vasculopathy is also seen in other transplanted solid organs such as the liver, kidney, and lung, known as vanishing bile duct syndrome, renal chronic rejection, and obliterative bronchiolitis, respectively. Thus, the potential benefit of Thymoglobulin® induction therapy may not be limited to CAV. Unfortunately, the utility and applicability of these retrospective studies are limited by either the complete lack of controls or the use of recipients from nearly a decade earlier as controls for comparison. It is well documented that the clinical practice of solid organ transplantation has changed significantly from decade to decade with a constant improvement in survival and decrease in rejection rate. With these severe limitations one cannot attribute these study outcomes to Thymoglobulin induction therapy. Therefore, a prospective, randomized, controlled trial is needed to definitively establish Thymoglobulin's potential efficacy in preventing CAV.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVES

(A) To describe between treatment groups the incidence of the composite primary endpoint of the development of de novo donor specific antibodies and ischemia on endomyocardial biopsy at 12 months post-transplantation

2.2 SECONDARY OBJECTIVES

To compare between treatment groups:

(A) Number of patients who develop cardiac allograft vasculopathy (CAV) (defined as a change ≥0.5mm in maximal intimal thickness (MIT) of the coronary arteries by intravascular ultrasound at 12 months as compared to baseline)
(B) correlations between significant changes in immune cell profiles and circulating antibodies, and their relation to any significant differences in clinical outcomes
(C) Number of patients who experience acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within first 12 months after transplantation
(D) Number of patients with biopsy proven cellular rejection ≥2R, biopsy proven antibody mediated rejection ≥AMR1 and any-treated rejection at 12 months post-transplantation
(E) Number of acute cellular, antibody-mediated, hemodynamic compromise and any-treated rejection episodes per patient within the first 12 months post-transplantation
(F) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection by the ISHLT biopsy grading scale in the first 12 months post-transplantation
(G) Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within the first 12 months
(H) the incidence of Primary Graft Dysfunction (PGD) in the first 24 hours post-transplant
(I) Patient and graft survival at 12 months post-transplantation
(J) the types and number of patients with both fatal and non-fatal infectious complications (especially CMV infection) within the first 12 months post-transplantation
(K) freedom from the development of circulating antibodies within the first 12 months post transplantation, where circulating antibodies include donor specific antibodies (DSA), non-specific antibodies, and non-human leukocyte antigen antibodies
(L) Change in coronary maximal intimal thickness, intimal area, intimal volume, vessel area, intimal index and percent atheroma volume (PAV) at matched sites by intravascular ultrasound at 12 months post-transplantation
(M) Maintenance doses of mycophenolate mofetil, tacrolimus, sirolimus, and cumulative dose of corticosteroids at 12 months post-transplantation
(N) Number of hospital days per patient, both during the transplant period and during the post-transplant period at 3 months, 6 months, and 1 year
(O) Number of patients requiring hospitalization by 3 months, by 6 months, or by 1 year post-transplantation

3. STUDY DURATION AND STUDY EARLY TERMINATION

3.1 PARTICIPANT COMPLETION

The study was initiated in September 2018 and enrollment will continue until October 31, 2023. The study will be completed 24 months after the last patient has entered. Study completion is expected by October 31, 2025

3.2 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator and/or the Medical Monitor no longer believes participation is in the best interest of the participant.

3.3 PREMATURE DISCONTINUATION OF STUDY DRUG

Study therapy will be prematurely discontinued for any participant if the participant has anaphylaxis, severe cytokine release syndrome or other hypersensitivity reactions. The management of severe infusion reactions should be dictated by the signs and symptoms of the reaction. Medical personnel with experience in treating anaphylaxis should be available. ATG administration in patients with a history of ATG anaphylaxis is not recommended.

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant, if the subject is judged non-compliant, or due to other safety concerns. Participants who become pregnant during the study treatment period will discontinue investigational agents.

3.4 STUDY STOPPING RULES

The principal investigator and Data and Safety Monitors will review safety data on an ongoing basis. If a safety concern arises, enrollment and randomization of participants in the trial will be suspended pending Data and Safety review. Subjects already enrolled will continue to be treated per protocol.

The criteria described below provide additional guidance for suspending trial enrollment/randomization based on the occurrence of selected adverse events. Because of the multiplicity of stopping guidelines each has been formulated to occur when the cumulative number of adverse event of concerns in either treatment group meets or exceeds a pre-defined threshold. Selected adverse events of concern and their thresholds in this trial are:

- Post-Transplant Lymphoproliferative Disorder (PTLD)
- Opportunistic infections
- Death

- Treated acute rejection with hemodynamic compromise

If a criterion is satisfied, the trial will be placed on hold pending Data and Safety review.

3.4.1 Post-Transplant Lymphoproliferative Disorder (PTLD)

The study will be placed on hold pending Data and Safety review if there is an occurrence of any case of PTLD reported in any randomized subjects at any time during the study.

3.4.2 Opportunistic Infections

Opportunistic infections are defined as tissue invasive CMV, pneumocystis, nocardia, aspergillus, invasive fungal infections (including invasive candida), toxoplasmosis, nocardiosis, zika, and west Nile virus. The study will be placed on hold pending Data and Safety review if the observed subject-based event rate in either group exceeds 30%. This rule will be met if the lower one-tailed 95% confidence limit on the estimated rate of opportunistic infections in either treatment group is greater than 0.30.

3.4.3 Death

Death from any cause is of concern early in the trial, when the threshold incidence rate of concern is 15%. That is, the rule will be met if the lower one-tailed 95% confidence limit on the estimated rate of death in either treatment group is greater than 0.15.

3.4.4 Rejection

Throughout the trial there would be safety concerns if the rate of subjects with any treated acute cellular rejection associated with hemodynamic compromise within 4 months of treatment exceeds 10%. This rule will be considered to have been met and the study will be placed on hold pending Data and Safety review if the lower one-tailed 95% confidence limit on the estimated acute rejection associated with hemodynamic compromise rate (within 4 weeks of treatment) is greater than 0.10.

4. CENTER OF STUDY

This study began as a single center study conducted at Cedars-Sinai Medical Center. As a multi-center study, participating centers include Cedars-Sinai Medical Center and Kaiser Permanente Medical Center.

5. SELECTION CRITERIA

5.1 TOTAL NUMBER OF PATIENTS

A total of 60 patients will be consented in the study.

5.2 RECIPIENT INCLUSION CRITERIA FOR STUDY ENTRY

The following criteria will apply to the patients enrolled in this study:

(1) Men and non-pregnant women must be 18 to 74 years old
(2) Women of childbearing potential must have a negative serum pregnancy test prior to transplantation.
(3) Men with a female partner of child bearing age and women of childbearing potential must use two reliable forms of contraception simultaneously. Effective contraception must be used before beginning study drug therapy, and for 4 months following discontinuation of study drug therapy.
(4) Subjects must be willing and capable of understanding the purpose and risks of the study, and must sign a statement of informed consent

5.3 RECIPIENT EXCLUSION CRITERIA FOR STUDY ENTRY

Patients meeting any one of the following criteria will be excluded from entering the study:

(1) Allergy to Thymoglobulin. Thymoglobulin is contraindicated in patients with history of allergy or anaphylaxis to rabbit proteins or to any product excipients, or who have active acute or chronic infections which contraindicate any additional immunosuppression
(2) Previous organ transplants
(3) Patients receiving multiple organs
(4) Patients with a BMI higher than 35
(5) Patients with PRA ≥ 25%
(6) Subjects with a Creatinine ≥ 2.0 mg/dl at time of transplant
(7) History of a psychological illness or condition which would interfere with the patient's ability to understand the requirements of the study
(8) HIV-1, chronic Hepatitis B, or chronic Hepatitis C infection, or a history of Chagas disease.
(9) Documented or strong suspicion for pre-operative active infection that has not yet been adequately treated with the recommended course of antimicrobial therapy
(10) Presence of any chronic myelosuppressive disease or agent that has resulted in either chronic leucopenia or chronic thrombocytopenia
(11) Active peptic ulcer disease and Active GI bleeding
(12) Patients who have received or require concomitant treatment with other investigational drugs within the past 30 days of transplant day (except for those listed in section 8.6 "Concomitant treatment")
(13) Patients with a history of AL amyloidosis (TTR amyloids) are permitted).

5.**4 RECIPIENT INCLUSION CRITERIA FOR RANDOMIZATION**

The following criteria will apply to the patients randomized in this study:

1) Women of childbearing potential must have a negative serum pregnancy test prior to transplantation.

5.5 RECIPIENT EXCLUSION CRITERIA FOR RANDOMIZATION

Patients meeting any one of the following criteria will not be randomized:

1. Patients requiring VAD upon completion of transplantation surgery.

2. White blood cell count ≤ 3000 /mm^3, or platelets ≤ 75,000/mm^3, or hemoglobin ≤ 7g/dL after completion of heart transplant

5.6 DONOR INCLUSION and EXCLUSION CRITERIA

Screening of donor hearts will follow Cedars Sinai standard of care protocol and given the shortage of suitable donor hearts for cardiac transplantation, COVID-19 positive hearts, HCV positive and DCD donor heart might be considered and accepted.

Decisions about whether to accept organs from donors who are HCV NAT positive must be made by transplant professionals and potential recipients through a shared decision-making process, on a case-by-case basis, when clinically appropriate.

6 DISEASE EVALUATION (EFFICACY AND SAFETY CRITERIA)

6.1 PRIMARY EFFICACY PARAMETERS

The following primary efficacy parameters will be measured in all patients:

(A) The incidence between groups of the development of de novo donor specific antibodies and ischemia on endomyocardial biopsy at 12 months post-transplant

6.2 SECONDARY EFFICACY PARAMETERS

To compare between groups:

(A) **Secondary Parameters of Efficacy:** Changes in percentages of various subsets of immune cells at pre transplant, 3 months and 6 months. Changes in percentages of various circulating antibodies at pre-transplant, 1 month, 3 months, 6 months, and 12 months after transplant.
(B) Freedom from cardiac allograft vasculopathy (CAV) (defined as a change ≥0.5mm in maximal intimal thickness (MIT) of the coronary arteries by intravascular ultrasound at 12 months as compared to baseline)

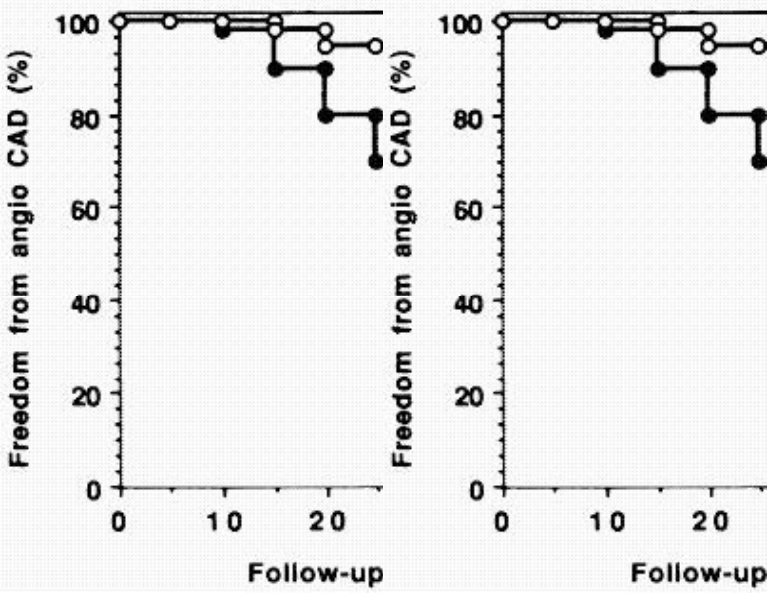
(C) Change in coronary maximal intimal thickness intimal area, intimal volume, vessel area, intimal index, and percent atheroma volume at matched sites by intravascular ultrasound at 12 months
(D) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within first 12 months after transplantation
(E) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection episodes per patient within the first 12 months post-transplantation
(F) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection by the ISHLT biopsy grading scale in the first 12 months post-transplantation
(G) Freedom from biopsy proven cellular rejection ≥2R, biopsy proven antibody mediated rejection ≥AMR1 and any-treated rejection at 12 months post-transplantation

(H) Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within the first 12 months post-transplantation
(I) Incidence of primary graft dysfunction (PGD) in the first 24 hours post-transplantation
(J) Patient and graft survival at 12 months post-transplantation
(K) The number of patients with both fatal and non-fatal infectious complications (especially CMV infection) within the first 12 months post-transplantation
(L) Freedom from the development of circulating antibodies within the first 12 months post-transplantation
(M) Average maintenance doses of Mycophenolate Mofetil, tacrolimus, and cumulative dose of corticosteroids at 12 months post-transplantation
(N) Average number of hospital days per patient, both during the transplant period and during the post-transplant period at 3 months, 6 months, and 1 year,
(O) Number of patients requiring hospitalization by 3 months, 6 months, or 12 months post-transplantation.
(P) Freedom from the development of circulating antibodies

6.3 DIAGNOSIS AND TREATMENT OF CAV

Although most institutions use the cardiac angiogram to diagnose CAV, the limitations and failure of angiogram are well known.[142] Intravascular ultrasound (IVUS) has been increasingly recognized as a sensitive tool to assess the anatomy of the epicardial coronary arteries. The procedure is performed at the time of the routinely scheduled angiogram and has been demonstrated to be safe and have reproducible findings with a correlation coefficient of 0.96 for inter-observer agreement.[143] An early concern about the safety of IVUS was the possibility that catheter irritation might accelerate the atherosclerotic process. These concerns have since been addressed. One study evaluating 86 patients undergoing IVUS demonstrated that **IVUS was not associated with the acceleration of arteriopathy in subsequent procedures.**[144] Another study in the *Journal of the American College of Cardiology* confirmed these findings.[145] The reported complications of IVUS occur in <1.1% of procedures and include focal coronary spasm, vessel dissection, guidewire entrapment, and acute occlusion which may result in myocardial infarction.[146]

A study within 6 to 8 weeks after transplant allows the measurement of the recipient's baseline atherosclerosis, which has been demonstrated to be not significantly changed at 2, 4, 6, or 8 weeks post-transplant.[147] Although several measurements are available to analyze IVUS images, maximal intimal thickness (MIT), defined as the greatest distance from the intimal leading edge to the external elastic membrane, has been shown to be the most clinically useful measurement because of its high reproducibility and its use in predicting outcome.[148] A maximal intimal thickness of ≥ 0.5 mm is the current widely accepted definition of atherosclerosis.[142] Follow-up imaging at 1 year provides important information regarding the development of CAV as the change in the first year IVUS results as compared to baseline can predict the course of the disease.[143] In two separate studies have demonstrated that a mean intimal thickening of >0.3 mm as measured by IVUS was associated with significantly worse 4 year overall survival (more death, myocardial infarction, and re-transplantation).[10,11] See the following two figures:



Plot showing freedom from development of subsequent angiographic coronary artery disease (angio CAD) in the sub-group of patients with normal angiograms at the time of intracoronary ultrasound according to intimal thickness of > 0.3 vs less or equal to 0.3 mm. In this analysis, the curve for the patient with intimal thickness of less or equal to 0.3 mm has been time-shifted by multiplying each data point by the ratio of a mean duration after transplantation in the two groups, i.e., 3.9/2.8 years. (open circle) indicates normal angiogram and intimal thickness less or equal to 0.3 mm; (closed circle), normal angiogram and intimal thickness > 0.3 mm[10].

Overall Mortality		Relative Risk	95% CI	*P*
IVUS	intimal thickness >0.3 vs ≤0.3 mm	4.7	1.3-14.7	.01
Angio: any vs no TxCAD		2.8	0.8-8.8	.09
Duration after transplant at ICUS study		1.07	0.9-8.1	.5

Abbreviations as in Table 1.

Relative Risks of Overall Mortality Associated With ICUS, Angiographic Findings, and Duration After Transplant in the Total Population[10]

Two other IVUS studies reported that a >0.5 mm increase in intimal thickening in the first year of transplant (termed "rapidly progressive intimal thickening") had significantly worse 5-year morbidity and mortality outcomes.[12,27]

A subset of transplant recipients will have a maximal intimal thickness measured at matched sites at 4 ± 2 weeks and again at 12 months ± 2 weeks post-transplant. Those whose change in maximal intimal thickness is ≥ 0.3 mm will be considered positive for CAV. Those who have a change ≥ 0.5 mm will be classified with "rapidly progressive CAV." As mentioned in Section 1.1.2 there is currently no treatment for CAV, only the slowing of its progression by the prompt treatment of rejection, modification of risk factors such as diabetes and smoking cessation, and the appropriate institution and maintenance of the agents that have been demonstrated to slow its progression (i.e. mycophenolate mofetil, vitamins C & E, calcium channel blockers, HMG CoA reductase inhibitors, everolimus/sirolimus, ganciclovir when appropriate, and aspirin).

6.4 DIAGNOSIS AND TREATMENT OF REJECTION

6.4.1 Diagnosis of Rejection

Acute cellular rejection may be diagnosed by an endomyocardial biopsy that has an ISHLT grade 2R or worse, the presence of hemodynamic compromise requiring treatment (with or without biopsy), or endomyocardial biopsy with positive immunohistochemistry stains for antibody-mediated rejection and significant hemodynamic compromise. Patients must be treated for rejection according to one of the following regimens in Table 1 (below and in Appendix A-2) depending on the ISHLT biopsy grade and the presence of hemodynamic compromise.

1) The date of onset of an episode of rejection will be defined according to the date of positive biopsy or treatment start date, whichever comes first
2) All suspected rejections must be biopsied and documented in the case report form
3) Endomyocardial biopsy to monitor allograft rejection will be performed according to Cedars-Sinai Medical Center standard of care and Kaiser Permanente's (for subjects repatriated to Kaiser), which typically occurs at the following time points:

   Weeks 2 and 4 (Day 14 and 28)
   Months 2, 3, 6 & 12 (Days 56, 90, 180 & 360)

4) The ISHLT Standardized Grading System will be used to assess cellular and antibody-mediated allograft rejection (see Appendix A-1)

   Weeks 2 and 4 (Day 14 and 28)
   Months 2, 3, 6 & 12 (Days 56, 90, 180 & 360)

6.4.2 Definition of Hemodynamic Compromise

Hemodynamic compromise is defined as **either**:

1) Ejection fraction of ≤ 30% or a 0.20 absolute decrease from baseline, and the need for inotropic agents

   OR

2) Fractional shortening ≤ 20% or a 25% decrease from baseline, and the need for inotropic agents
   PLUS

3) Need for inotropic agents due to a Cardiac Index (CI) < 2.0 L/min/m2 or a 25% decrease from baseline

6.4.3 Treatment of Cellular Rejection

All suspected rejections must be biopsied and documented in the case report form. Rejection episodes will be treated according to the regimens specified in Table 1 (and Appendix A-2).

A follow-up biopsy must be done within 2 weeks after the end of treatment for rejection. Resolution of rejection will be defined when no further treatment is required. If the same or additional treatment is required for the current rejection (according to the regimen specified in Table 1), the rejection is still considered an ongoing episode. A follow-up biopsy must be done within 2 weeks after the end of this treatment. A second episode of rejection may not be counted until the first episode has resolved.

Rejection episodes which fail to respond to the specified treatment course may be treated with the same course or treated with the next regimen as specified in the Table 1. For example, a Grade 2R rejection episode treated with I.V. corticosteroids which fails to resolve may be treated with another course of corticosteroids as specified in Table 1 under 2R with no hemodynamic compromise or as specified in Table 1 under 2R with hemodynamic compromise. Immunosuppressants used to treat acute allograft rejection will be recorded on the case report form.

Table 1	**Hemodynamic compromise and/or clinical symptoms**	
ISHLT biopsy grade	**Absent**	**Present**
Grade 1R Mild	No treatment	1-2mg/kg oral corticosteroids for 3-5 days followed by 5-10 day taper or immediate return to Baseline oral corticosteroids and/or High dose i.v. corticosteroids with or without ATG therapy for 5 days
Grade 2R Moderate	1-4mg/kg oral corticosteroids for 3 to 5 days followed by 5 to 10 day taper or immediate return to baseline oral corticosteroids or 500mg to 1 g i.v. corticosteroids for 3 days followed by immediate return to baseline oral corticosteroids	500mg - 1 gm i.v. corticosteroids for 3 days ± 5 days of ATG therapy followed by taper or immediate return to Baseline oral corticosteroids and/or High dose i.v. corticosteroids with or without ATG therapy for 5 days
Grade 3R Severe	High dose i.v. corticosteroids +5 days of ATG therapy	High dose i.v. corticosteroids with or without ATG therapy for 5 days

*Hemodynamic compromise is defined in section 6.4.2

6.4.4 Treatment of Antibody Mediated Rejection

Antibody mediated rejection (AMR) as defined by the 2013 ISHLT pathologic grading system (see appendix A-1) will be treated according to the standard of care.

6.5 DIAGNOSIS OF ISCHEMIA-REPERFUSION INJURY

Ischemia-reperfusion injury (IRI) is hypoxic and anoxic tissue injury from inflammation during reperfusion after ischemia. The diagnosis of IRI is made on the basis of findings on the endomyocardial biopsy. The endomyocardial biopsy forms the standard of care to monitor the

allograft for rejection and will be performed according to Cedars-Sinai Medical Center standard of care, which typically occurs at the following time points:

Weeks 2 and 4 (Day 14 and 28)
Months 2, 3, 6 & 12 (Days 56, 90, 180 & 360)

IRI is characterized on biopsy by myocyte necrosis and/or regions of myocyte dropout. Mild neutrophil infiltrates or no inflammation is seen, which contrasts with the mononuclear and eosinophilic infiltrates seen in acute cellular rejection. Signs of IRI are routinely screened for as part of the standard of care and reported as either:

- Ischemic changes: up to 6 weeks post-transplant
- Ischemic changes: late; related to allograft coronary disease

6.6 PHARMACOKINETIC/PHARMACODYNAMIC PARAMETERS

No pharmacokinetic/pharmacodynamic evaluations will be performed.

7. STUDY DESIGN

7.1 DESIGN

This is a randomized, controlled, multi-center study to evaluate the efficacy of Thymoglobulin induction therapy in combination with Mycophenolate Mofetil, tacrolimus, and steroids in the prevention of CAV. Approximately half of the patients will be randomized to receive a total of 5 doses of Thymoglobulin during the study.

For the subjects randomized to receive the study drug, the first two doses of Thymoglobulin will be administered at 1.5 mg/kg via intravenous infusion over 8 hours immediately upon arrival to the ICU post-operation [day 1 (POD#0) and day 2 (POD #1}. Subsequent doses of 1.5 mg/kg will be administered on days 3, 4, and 5 via IV infusion over 4-8 hours.

The subjects will be consented onto the study while on the UNOS waitlist. The screening assessments may be drawn after consent is obtained and any time prior to transplant. Patients who qualify for the study will be administered the following treatment regimens:

Table of Study Medication Administration

Medication	POD#0/ Day 1	POD#1/ Day 2	POD#2/ Day3	POD#3/ Day 4	POD#4/ Day 5	POD #30	POD #90	POD #180	POD #365
Thymoglobulin/ No Induction or Control*	1.5 mg/ kg over 8 hrs within 24 hours post Tx	1.5 mg/ kg over 8 hrs	1.5 mg/ kg over 4-8 hrs	1.5 mg/ kg over 4-8 hrs	1.5 mg/ kg over 4-8 hrs	Stopped after POD#4			
Mycophenolate Mofetil/MMF	1.5 g bid	Continue & adjust dose as indicated by side effects							
Tacrolimus	1 to 4 mg bid	Titrate to trough	Titrate to trough	Titrate to trough	Trough 10-15 ng/mL	Trough 8-12 ng/mL	Trough 5-10 ng/mL	Trough lowered to 5-10 ng/mL if no rejection	
Corticosteroids	125 mg IV Solumedrol q12 hrs x 3 doses	Prednisone 1 mg/kg/ day po divided into bid doses	Taper by 10 mg qd	Taper to 10 mg po bid		Taper by 2 mg q 1-2 wks to 5 mg po bid	Taper by 2 mg q 2 wks until 5 mg po qd	Discontinue steroids	
HMG CoA Reductase Inhibitor**	First dose initiated within 2 weeks of surgery	Dose titrated up as tolerated	Continued as tolerated						
Vitamin C**	500 mg qd	Continue as tolerated							
Vitamin E**	400 IU qd	Continue as tolerated							
Aspirin**	81 mg qd	Continue as tolerated							

* First dose within 24 hours post transplant

** Dosages will be prescribed according to institution's standards

Patients will be seen at Screening, Post op days Day 1-5, Day 7, 14, 21, 28, 42, 56, 90, 120, 150, 180, 240, 300 and 365. Laboratory parameters, diagnostic tests and clinical evaluations will be obtained as needed

All patients, including premature withdrawals, will be followed for a total of 12 months post study start date for death, re-transplantation, acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection episodes regardless of whether they are receiving study medication.

7.2 STUDY PROCEDURES

7.2.1 Screening/Baseline

Prospective patients will be identified by the principal investigator and written informed consent must be obtained before the performance of any screening procedures. Patients will be screened within 48 hours of entry into the study. The following standard of care data will be extracted from the medical record:

(A) Medical history
(B) Body Weight
(C) Laboratory tests:
  (1) CBC with differential, platelet count
  (2) Chemistry panel
  (3) Pregnancy test (serum) must be done prior to transplantation. Urine test is allowed in addition to serum test in patients where serum results are delayed.
  (4) Panel of reactive antibodies (PRA)
  (5) HLA (DR) mismatch
  (6) CMV and EBV serologic status of the recipient and donor

Blood and platelet loss will be monitored during the post-transplantation period and the following will be recorded:

(a) Number of PRBC and platelet transfusions
(b) Amount of blood lost during first 48 hours post-transplant

7.2.3 Clinical Evaluations

The following standard of care data will be extracted from the medical record. Please see the Schedule of Assessments for a complete outline of study visits and visit specific procedures.

(A) Clinical Assessment—clinical evaluation of patients' signs or symptoms of rejection, adverse events, infections, and malignancies (throughout entire inpatient stay post-transplantation and at every clinic visit)
(B) Endomyocardial biopsy
Any patient who prematurely discontinues from the study will continue to have endomyocardial biopsies as is currently the standard for routine post-transplant care
(C) Vital signs, including blood pressure, pulse, temperature (every visit) and body weight (all visits)
(D) Laboratory evaluations:
  a. CBC with differential, platelets, electrolytes, creatinine, BUN, and TACROLIMUS if completed per standard of care or as needed.

b. Immune profile and circulating antibodies

(E) Concomitant medication: All concomitant medications, particularly immunosuppressive therapy and prophylactic therapy should be reviewed with the patient at every visit and recorded on the CRF (all visits)

(F) Adverse events (all visits). See section 11.4.2 for definition of an adverse event

(G) Intravascular ultrasound

(H) Echocardiogram

7.3 RANDOMIZATION PROCEDURE AND ASSIGNMENT TO TREATMENT GROUP

Patients consented to the study and meeting study entry and randomization inclusion and exclusion criteria will be randomized in a 1:1 ratio to either Thymoglobulin® or No Induction or Control during the immediate post transplantation period.

Randomization will be implemented through RedCap by Cedars-Sinai. Patients who are randomized will receive a unique drug randomization number that corresponds to his/her unique study identification number.

8. STUDY MEDICATION

8.1 DRUG NAME, FORMULATION, & STORAGE

HOW SUPPLIED

Thymoglobulin is available as sterile, lyophilized powder to be reconstituted with Sterile Water for Injection, USP (SWFI). Each package contains a 10 mL vial freeze-dried Thymoglobulin (25 mg) NDC# 58468-0080-1.

Reconstituted Thymoglobulin is physically and chemically stable for up to 24 hours at room temperature; however, room temperature storage is not recommended. As Thymoglobulin contains no preservatives, reconstituted product should be used immediately.

8.2 PACKAGING AND LABELING

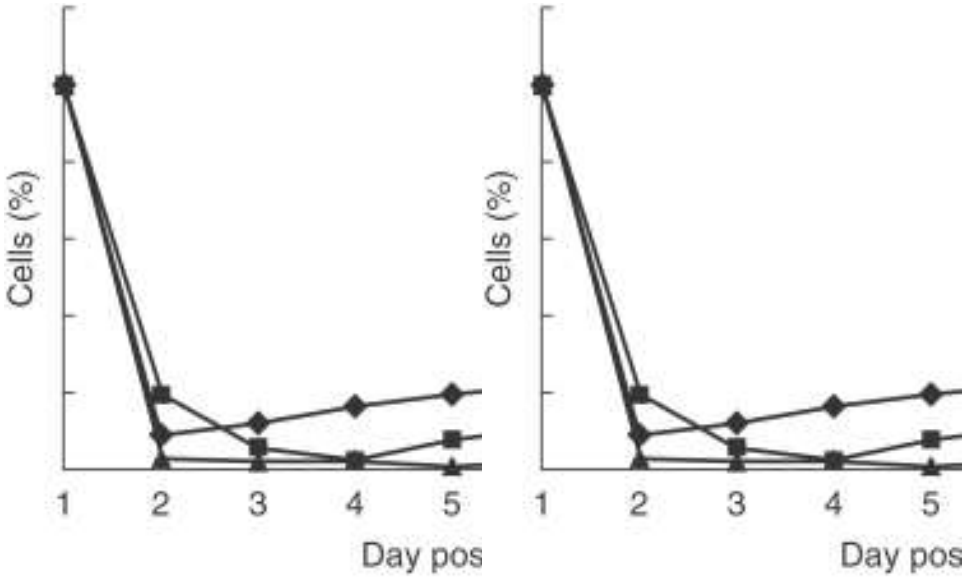
The Thymoglobulin® will be supplied by Genzyme and packaged in 25 mg vials. Thymoglobulin® will be shipped to the Cedars-Sinai Investigational Pharmacy from Sanofi. The study drug will be labeled per CFR Title 21 312.6: “Caution: New Drug—Limited by Federal (or United States) law to investigational use,” and will contain the protocol name/# on the label.

As per the manufacturer’s guidelines the study drug will be stored between 2 to 8 degrees C (36 to 46 degrees F) away from light. Study drug will not be frozen or used after the expiration date indicated on the label. Reconstituted vials of Thymoglobulin® will be used within 4 hours. Infusion solutions of Thymoglobulin® must be used immediately. Any unused drug remaining after infusion must be discarded.

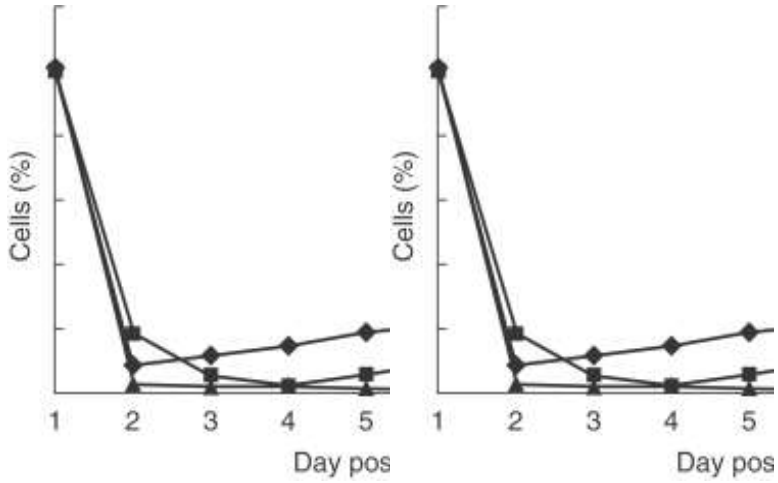
8.3 STUDY TREATMENT

8.3.1 Rationale for Dose Selection

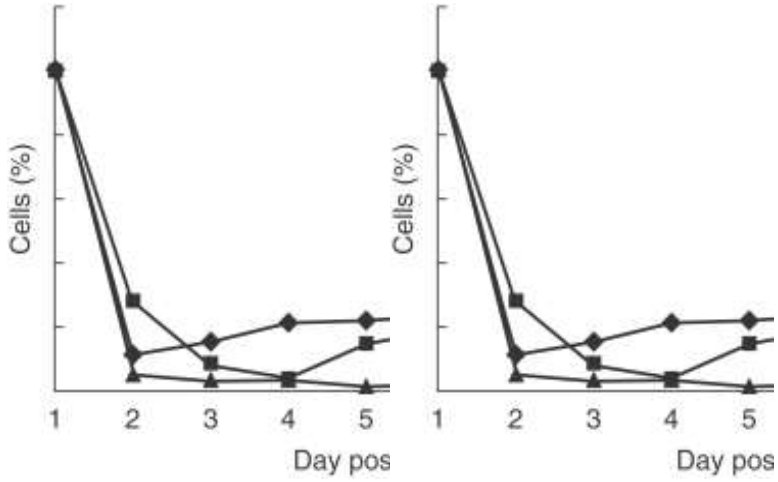
Neither a dose response nor a time course study has been performed on cardiac transplant patients with Thymoglobulin®. Thus, we have selected a dosing regimen of 1.5 mg/kg/day intravenously for 5 days based on some scientific data as well as current popular methodology. Thymoglobulin® has been administered to over 40,000 renal and cardiac transplant recipients in Europe since 1985 and the most popular dose currently used for induction purposes is 1.5 mg/kg/day for 4 days[149]. As mentioned above in Section 1.2.2 no correlation has been seen between treatment outcomes and either active or total Thymoglobulin® concentrations in renal transplant patients who were dosed at 1.5 mg/kg/day for 6-14 days[119]. This implies that there is probably no benefit to more than 6 days of therapy. A number of studies measuring various T-cell responses to patients receiving Thymoglobulin®, have consistently demonstrated significantly lower absolute T-cell counts as well as significantly lower CD3, CD2, CD4, CD8, and CD19 by day 5 of administration[120,150-153], suggesting that the optimal dosing time course is probably 5 or 6 days.



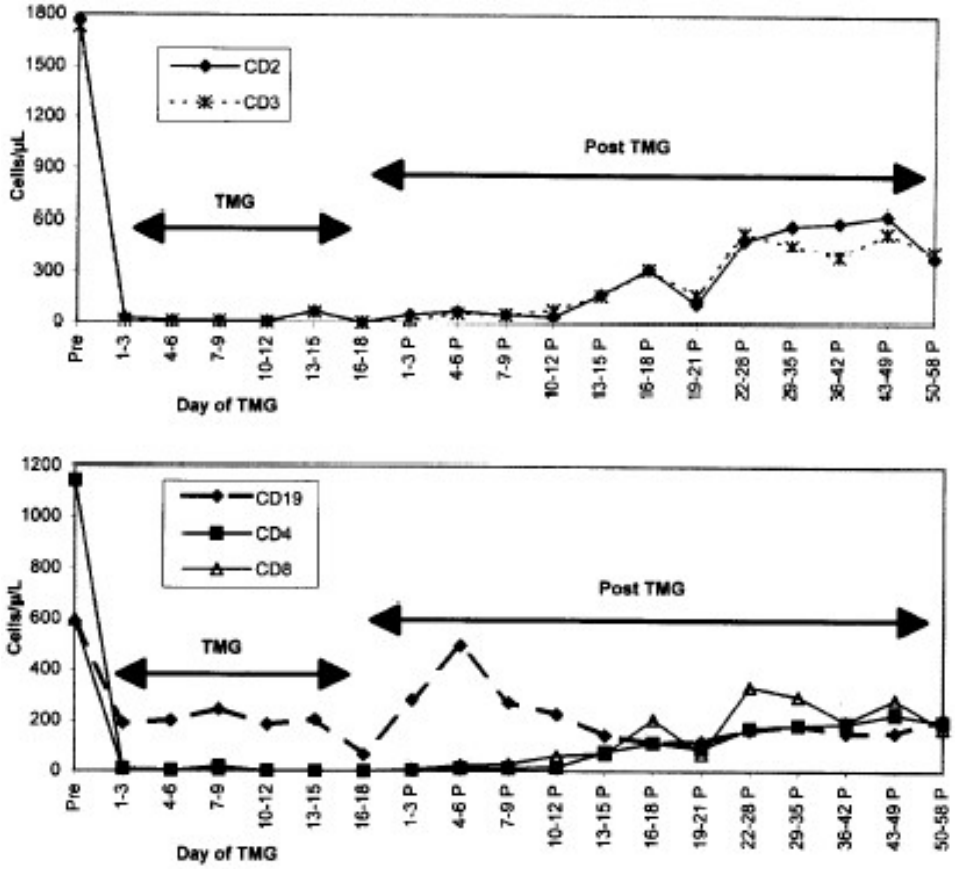
The effect of thymoglobulin (Thy, ▲), anti-thymocyte globulin (ATG, ◆) or Minnesota anti-lymphoblast globulin (ALG, ▪) **on CD3 T-cells** over 10 days of flow cytometric analysis. Data are expressed as the percentage of cells remaining relative to the preinduction level, represented on day 1 at 100%. Note that **Thy was administered for only the first 5 days** while ATG and ALG were administered over the full 10 days[154]



The effect of thymoglobulin (Thy, ▲), anti-thymocyte globulin (ATG, ●) or Minnesota anti-lymphoblast globulin (ALG, ■) on CD4 T-cells over 10 days of flow cytometric analysis. Data are expressed as the percentage of cells remaining relative to the preinduction level, represented on day 1 at 100%. Note that **Thy was administered for only the first 5 days** while ATG and ALG were administered over the full 10 days.



The effect of thymoglobulin (Thy, ▲), anti-thymocyte globulin (ATG, ●) or Minnesota anti-lymphoblast globulin (ALG, ■) **on CD8 T-cells** over 10 days of flow cytometric analysis. Data are expressed as the percentage of cells remaining relative to the preinduction level, represented on day 1 at 100%. Note that **Thy was administered for only the first 5 days** while ATG and ALG were administered over the full 10 days[154]



Refer to above paragraph: Depletion of CD2, CD3, CD4, CD8, and CD19 positive cells was rapid, with counts falling to less than 30 cells/pL, and was sustained for several weeks after Thymoglobulin®[155].

The Thymoglobulin® package insert recommends a dose of 1.5 mg/kg/day for 7-14 days in the treatment of acute renal graft rejection. Despite this recommendation, there are numerous studies in acute renal rejection in which Thymoglobulin® was administered either at less than 1.5 mg/kg/day and/or less than 7 days of therapy without a difference in clinical outcome.[106,121,123,125,154,156] However, one must keep in mind that the main purpose of Thymoglobulin® induction therapy in renal transplantation is to be able delay the initiation of the nephrotoxic calcineurin inhibitors while the renal graft is recovering and at the same time obtain effective "nephro-safe" immunosuppression. In most of the renal transplantation protocols Thymoglobulin is administered for an unspecified, open ended number of days until the renal graft has recovered from either surgery or rejection, which is usually more than 7 days. Thus, the recommendation of 7-14 days seems justified for use in renal transplantation. **This study will incorporate Thymoglobulin® in addition to the non-delayed introduction of triple immunosuppressive therapy immediately post-transplant with the objectives of reducing the incidence of acute rejection and CAV without increasing the risk of infection.** Thus, for the purposes of this study, the recommended 7-14 days of Thymoglobulin® therapy probably does not apply.

8.3.2 Dosage Regimen and Dose Adjustment

A total of 5 doses of Thymoglobulin® will be administered per recipient at 1.5 mg/kg/dose. The appropriate dose should first be reconstituted. Transfer the contents of the calculated number of Thymoglobulin® vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL). Mix the solution by inverting the bag gently only once or twice. The first two doses should be administered over a 8 hour continuous intravenous central infusion. The reconstituted/diluted medication may be stored for up to 4 hours at 2 to 8 degrees C before administration. Each patient should be premedicated with 650 mg of acetaminophen and 25 or 50 mg of diphenhydramine (depends on the patient's weight) and Methylprednisolone 125 mg IV approximately 60 minutes before each infusion of Thymoglobulin®. The dose of diphenhydramine should be 25 mg for any patient less than 60 kg. If the patient is 60 kg or greater , a dose of 50mg is preferred.

The first dose of Thymoglobulin® will be administered at a dosage of 1.5 mg/kg IV upon arriving to the Intensive Care Unit after transplantation (called POD Day 0/Day #1) along with the first dose of IV methylprednisolone. The first dose must be administered within 24 hours after transplantation in order for the patient to continue in the study. The subsequent 4 doses also at 1.5 mg/kg will be administered on days 2, 3, 4, and 5 every 24 hours from the start of the first dose.

There has been one prospective, randomized, clinical trial of intraoperative versus postoperative initiation of Thymoglobulin® therapy in renal transplant recipients where intraoperative administration was associated with significantly less initial delayed graft function[106]. Although there may be a hypothetical advantage to administering Thymoglobulin® intraoperatively, there is concern that if side effects (such as anaphylaxis, see Section 10) were to occur intraoperatively or during transport, the limitations of the intra-operative setting would preclude the prompt and highly skilled intervention that is needed to treat the adverse event(s). Therefore, Thymoglobulin® induction will be initiated immediately upon arrival to the Intensive Care Unit in this study.

8.3.3 Dose Modification

Any toxicity associated with the Thymoglobulin® infusion that is also considered by the physician to be a major threat to the patient mandates discontinuation of the antibody infusion and/or discontinuation of the course of therapy.

The two main concerns are the development of leucopenia (WBC <2,000 cells/mm^3) and thrombocytopenia (platelet count <50,000 cells/mm^3). Doses will be modified as outlined in the tables below (obtained from Thymoglobulin® package insert):

Absolute WBC count	**Thymoglobulin® dose mg/kg/day**
>3,000 cells/mm3	1.5 mg/kg/day
2,000-3,000 cells/mm^3	0.75 mg/kg/day
<2,000 cells/mm3	Hold until WBC count >2,000

Platelet count	**Thymoglobulin® dose mg/kg/day**
>75,000 cells/mm^3	1.5 mg/kg/day

50-75,000 cells/mm3	0.75 mg/kg/day
<50,000 cells/mm3	Hold until platelet count >50,000

Holding Thymoglobulin treatment will be considered only if persistent and severe thrombocytopenia (< 50,000 cells/mm3) occurs or leukopenia (< 2,000 cells/mm3) develops.

White blood cell and platelet counts will be monitored during and after Thymoglobulin therapy. Once the blood counts have recovered the recipient will resume therapy.

Chills and fever may occur but generally can be controlled by the administration of diphenhydramine hydrochloride (25 to 50 mg IV every 6 hours as needed) and acetaminophen (325 to 650 mg PO every 4 hours as needed). For severe fever and chills, meperidine (25 mg IV every 4 to 6 hours as needed) may also be required. When any of the above non-life-threatening toxicities occur, the infusion should be interrupted until symptoms subside and then restarted at a slower rate.

Respiratory distress, pain the flank or back, or hypotension may be signs of anaphylaxis. The infusion should be discontinued and not resumed, and the course of treatment should be terminated. The above reactions must be entered on the case report form, and the patient will continue to be followed for safety for the duration of the study.

Other minor toxicities can usually be managed with symptomatic treatment and slowing of the infusion.

8.3.4 Route of Administration

Thymoglobulin® should be used under strict medical supervision in a hospital setting, and patients should be carefully monitored during the infusion. The first dose should be infused within 24 hours post-transplant into a high-flow vein. Follow the manufacturer's instructions for the infusion administration set. Infuse through a 0.22 micrometer filter into a high-flow vein.

Although ATG was administered intramuscularly in earlier studies, currently the intravenous route is universally used, as according to the guidelines provided by Genzyme (see package insert). Intravenous Thymoglobulin® has been demonstrated to be safe for use in the ambulatory setting.[122]

The drug will be administered via constant intravenous infusion within 24 hours (first dose) or 4 hours (subsequent doses). A central line will be used for administration of study drug.[122]

8.3.5 Treatment Time Course

All patients randomized to study drug will receive 5 doses of Thymoglobulin® unless an adverse event warrants dose reduction or temporary discontinuation. The necessity of any dose adjustment should be discussed with the medical data monitor. The first dose will be administered upon arrival to the Intensive Care Unit.

8.4 DISPENSING AND ACCOUNTABILITY OF STUDY MEDICATION SUPPLIES

Although Thymoglobulin® will be administered as standard of care, a drug administration log will be kept by the Study Coordinator and will identify the patient and the amount of medication administered to the patient.

8.5 CONCOMITANT TREATMENT

Concomitant medications, including nitric oxide for pulmonary hypertension, will be permitted as necessary.

8.5.1 Concomitant Immunosuppressive Therapy

Standard supportive immunosuppressive therapy will be prescribed to all patients enrolled in the study. The standard concomitant therapy is described below (see Table of Study Medication Administration in Section 7.1):

1) Mycophenolate mofetil: 3.0 grams divided bid begun post-transplant, either IV or po as tolerated by patient. Initial dose must be given within 24 hours post-transplant. Dosing will be titrated based on recipient's body size and any adverse side effects
2) TACROLIMUS: Doses of 1-4 mg bid either IV or po will be administered on post-operative day #0 to achieve a target trough level of 10-15 ng/mL by post-operative day #5. Target trough levels are 10-15 ng/mL for post-operative days #1-90 and 5-10 ng/mL thereafter. In patients with creatinine >0.2 mg/dL, delayed initiation of tacrolimus to post-operative day 3 will be done to allow recovery of renal function.[157]
3) Corticosteroids: 125 mg IV methylprednisolone immediately post-operatively x 3 doses q12hrs, then switching to oral prednisone at 1.0 mg/kg/day po divided into bid doses that are rounded off to the next higher 5 mg increment. For example, a 76 kg person should be dosed at 38 mg po bid, which rounded off to the next 5 mg increment would be 40 mg po bid. (Equivalent dosing via an alternative route may be used if po is not tolerated or contraindicated). Prednisone will be tapered by 10 mg qd until the dose of 10 mg po bid is reached.

Patient will then be tapered by the following schedule (as per our institutional protocol) as tolerated by lack of evidence for rejection:

Continue 10 mg po bid until post-operative day #30
Taper by 2 mg every 1-2 weeks to achieve 5 mg po bid by day #90
Taper by 2 mg every 2 weeks to achieve 5 mg po qd by day #180
Steroids then discontinued

8.5.2 Other Concomitant Therapy

1) HMG CoA Reductase Inhibition: All patients will receive lipid lowering/anti-inflammatory therapy, either pravastatin or simvastatin, as per our institutional protocol. Patients will receive one dose daily initiated at the time of oral administration or within 1-2 weeks of surgery. Dosages will be titrated up to a maximum dose 40 mg as tolerated by the patient. If total cholesterol still exceeds 200 mg/dL at 40 mg of pravastatin, a stronger agent such as atorvastatin may be substituted and/or ezetimibe added.

2) Calcium channel blockers or ACE inhibitors: All patients who become hypertensive post-transplant will receive a calcium channel blocker and/or an ACE inhibitor (amlodipine or nifedipine extended release), the dosage of which will be titrated according to the patient's blood pressure.
3) Antioxidants: All patients will be maintained on 400 IU of vitamin E and 500 mg vitamin C daily as according to our institutional protocol.
4) Aspirin: All patients will receive at least 81 mg of aspirin daily as according to our institutional protocol.

8.5.3 Mycophenolate Mofetil Dose Adjustment for Adverse Events

Mycophenolate mofetil is known to cause leucopenia. In the event of leucopenia (defined as a WBC count <2,000 cells/mm^3) or another known adverse side effect of mycophenolate mofetil, mycophenolate mofetil doses may be reduced or interrupted. Patients will not be withdrawn from the study because of extended mycophenolate mofetil dose reductions or interruptions. However, the investigator will attempt at his/her discretion to return the patient to the full maintenance dose upon resolution of the adverse event. After a dose interruption, every effort will be made within 14 days, unless medically warranted, to re-instate mycophenolate mofetil in increments until the recommended mycophenolate mofetil dose is achieved. If this is not possible, then Mycophenolate mofetil will be increased in stepwise increments of 250 mg until an optimal tolerable dose is found.

8.5.4 Prophylactic Therapy

All patients will also be prescribed prophylactic treatment as follows:

1) Antimicrobial prophylaxis
    a) Bacterial and Pneumocystis carinii pneumonia prophylaxis: Bactrim DS 2-4 tablets per week for 1 year. For patients allergic to sulfa, dapsone at 50-100 mg orally, administered daily, or aerosolized pentamidine, 300 mg per month will be substituted for Bactrim DS.
    b) Fungal prophylaxes: Nystatin 5000 U qid or Mycelex troches for a minimum of 6 months
2) CMV prophylaxis: (CMV disease defined in Appendix B)

All recipients who receive an organ from a CMV positive donor will be treated with 2 weeks of IV ganciclovir: 5-10 mg/kg IV ganciclovir for 14 days followed by oral ganciclovir (Cytovene) for a total of 6 months of therapy.
Recipients that are CMV positive, who receive a CMV negative donor heart will receive ganciclovir/valganciclovir for 3 months.

All concomitant medications will be reported in the case report form including start and stop dates.

8.5.5 Drug Interactions

The following medications have been administered in clinical trials with Thymoglobulin® without incremental increase in adverse reactions: daclizumab, cyclosporine, mycophenolate mofetil, ganciclovir, azathioprine, corticosteroids, sirolimus, and tacrolimus.

9. PREMATURE WITHDRAWAL

Patients who prematurely discontinue treatment with the study therapy regimen will remain in the study and follow the visit schedule. Thymoglobulin® may be prematurely discontinued/terminated for any patient for life threatening reactions. The study therapy regimen may also be prematurely discontinued for any participant if the investigator believes that the treatment is no longer in the best interest of the subject, if the subject is judged non-compliant, or due to safety concerns.

9.1 FOLLOW-UP

All patients (including those who prematurely discontinued) will be followed for a total of 12 months for the following data: death and re-transplantation, development of malignancies, and acute rejection episodes.

If the reason for removal of a patient from the study is an adverse event or an abnormal laboratory test result, the principal specific event or test will also be recorded on the case report form.

10. WARNINGS AND PRECAUTIONS

Thymoglobulin® adverse events are generally manageable or reversible (see table below). Thymoglobulin® should only be used by physicians experienced in immunosuppressive therapy. Medical surveillance is required during Thymoglobulin® infusion. Serious immune-mediated reactions have been reported with the use of Thymoglobulin® and consist of anaphylaxis and cytokine release syndrome (CRS). Fatal anaphylaxis has been reported. If anaphylaxis occurs, the infusion should be terminated immediately. Medical personnel should be available to treat patients who experience anaphylaxis. Emergency treatment such as 0.3 mL to 0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously and other resuscitative measures including oxygen, intravenous fluids, antihistamines, corticosteroids, pressor amines, and airway management, as clinically indicated, should be provided. Thymoglobulin® or other rabbit immunoglobulins should not be administered again for such patients. Severe, acute infusion-associated reactions are consistent with CRS which is attributed to the release of cytokines by activated monocytes and lymphocytes. Severe acute CRS can cause serious cardiorespiratory events and/or death. Thrombocytopenia or neutropenia may result from cross-reactive antibodies and is reversible following dose adjustments (see Section 8.3.2.1). During Thymoglobulin® therapy, monitoring the lymphocyte count (i.e., total lymphocyte and/or T-cell subset) may help assess the degree of T-cell depletion. For safety, WBC and platelet counts should also be monitored.

Thymoglobulin® infusion may produce fever and chills. To minimize these, the first two doses should be infused over a minimum of 8 hours and the rest of the doses(3-5) should be infused 4-8 hours into a high flow vein . Also, premedication with corticosteroids (methyl prednisone), acetaminophen, and/or an antihistamine and/or slowing the infusion rate may reduce reaction incidence and intensity. Adverse reactions more frequently reported following Thymoglobulin® infusion than following Atgam® in a phase III randomized controlled trial:

Side effects	Thymoglobulin® (n=82)# pts (%)
Chills	**47 (57.3)**
Leucopenia	**47 (57.3)**
Headache	**33 (40.2)**
Abdominal pain	**31 (37.8)**
Hypertension	**30 (36.6)**
Nausea	**30 (36.6)**
Dyspnea	**23 (28.0)**
Hyperkalemia	**22 (26.8)**
Myalgia	**16 (19.5)**
Insomnia	**16 (19.5)**
Hypotension	**13 (15.9)**
Rash	**11 (13.4)**
Sweating	**11 (13.4)**
Malaise	**11 (13.4)**
Acne	**10 (12.2)**
Overdose	**5 (6.1)**

Infections, reactivation of infection, and sepsis have been reported after Thymoglobulin® administration in combination with other immunosuppressive agents. Appropriate antiviral, antibacterial, antiprotozoal, and/or antifungal prophylaxis is recommended. Infections over a 3 month follow-up in the Phase III trial[109] are summarized in the table below, but the lack of a proper control group makes it difficult to credit Thymoglobulin® as the sole cause of these infections.

Infections (Thymoglobulin® n=82)

BODY SYSTEM **Preferred Term**	% of patients	# of patients	Total Reports
BODY AS A WHOLE	36.6	30	36
Infection	30.5	25	26
Other	17.1	14	15
CMV	13.4	11	11
Sepsis	12.2	10	0
Moniliasis	0	0	
DIGESTIVE	6.1	5	5
Gastrointestinal Moniliasis	4.9	4	4
Oral monoliasis	3.7	3	0
Gastritis	1.2	1	1
RESPIRATORY	0.0	0	0
Pneumonia	0.0	0	0
SKIN	4.9	4	4

Herpes simplex	4.9	4	4
UROGENITAL	18.3	15	15
Urinary tract infection	18.3	15	15
Vaginitis	0.0	0	0
NOT SPECIFIED	0.0	0	0

The safety of immunization with attenuated live vaccines following Thymoglobulin® therapy has not been studied; therefore, immunization with attenuated vaccines is not recommended for patients who have recently received Thymoglobulin®.

Use of immunosuppressive agents, including Thymoglobulin®, may increase the incidence of malignancy, including lymphoma or lymphoproliferative disorders (which may be virally mediated). These events have been associated with fatal outcome. The carcinogenic and mutagenic potential of Thymoglobulin® and its potential to impair fertility have not been studied.

Reactions at the infusion site can occur and may include pain, swelling, and erythema.

Thymoglobulin® can stimulate the production of antibodies which crossreact with rabbit immune globulins. Thymoglobulin® has not been shown to interfere with any routine clinical laboratory tests which do not use immunoglobulins. Thymoglobulin® may interfere with rabbit antibody-based immunoassays and with cross-match or panel-reactive antibody cytotoxicity assays.

The safety of Thymoglobulin® in pregnant women and nursing mothers has not yet been tested. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Thymoglobulin® is administered to a nursing woman. Women of childbearing potential must have a negative serum pregnancy test prior to transplantation. Urine test is allowed in addition to serum test in patients where serum results are delayed. Effective contraception must be used by women of childbearing potential before beginning Thymoglobulin® therapy and during therapy even where there has been a history infertility (unless due to hysterectomy) and up to 4 months following discontinuation of study medication. Two reliable forms of contraception must be used simultaneously unless abstinence is the chosen method.

If pregnancy occurs during the treatment or within the 12 months following discontinuation of study medication, the physician and patient should discuss the desirability of continuing the pregnancy. All patients who become pregnant while receiving study medication or for 4 months following discontinuation of the study medication must be followed until termination or completion of the pregnancy. The investigator must complete an SAE report in addition to completing the Pregnancy Outcome Page in the case report form.

10.1 OVERDOSE OR EXAGGERATED RESPONSE

Thymoglobulin® overdosage may result in leukopenia (including lymphopenia and neutropenia) or thrombocytopenia, which can be managed with dose reduction. The five doses of Thymoglobulin® will be administered over five days in all patients while they are still in the hospital as the average post-cardiac transplant patient at our facility stays a minimum of 6 days. Thus,

accidental overdosing could occur in the setting of a nursing error, where the wrong dose is calculated and administered. There is no antidote for Thymoglobulin® overdose. Treatment would consist of prompt cessation of the drug, close clinical observation, and monitoring of the blood count for profound thrombocytopenia, neutropenia, anemia, or lymphocytopenia and their expected normalization upon discontinuation of therapy. Platelet and blood transfusions may be necessary should the hemoglobin fall below 8 g/dL or the platelet count fall below 50,000 cells/mm^3 and the patient is actively bleeding. However, the benefit of a transfusion will need to be weighed against the risk of immune activation, and the decision to transfuse should be made by the transplant attending on a case by case basis. Once the blood counts have recovered, the recipient will resume therapy based on the dosing table provided in Section 8.3.2.1.

11. SAFETY PARAMETERS

During the study, safety assessments will include:

1) Clinical assessments, including weight and vital signs
2) Incidence of adverse events, malignancies, opportunistic infections, and premature withdrawal due to adverse events
3) Collection of selected concomitant medications and immunosuppressive therapies
4) Laboratory assessments
5) Graft loss and death
6) Vital signs (temperature, respiration, blood pressure, and heart rate) will be noted preinfusion, immediately post-infusion, and 15 minutes post-infusion of Thymoglobulin® and reported on the appropriate case report form.

11.1 MEDICAL HISTORY AND CLINICAL EVALUATIONS

A complete medical history will be performed during screening. This assessment will include age, sex, race, weight, the etiology of the cardiac disease, information on ABO matching of recipient and donor, results of panel reactive antibody (PRA)—both the highest and most recent assessment, HLA (DR) mismatch, CMV and EBV serologic status of the recipient and donor, Hepatitis B and C, cold ischemic time, and age of donor organ. Patients will be evaluated during the study for signs and symptoms including adverse events, opportunistic infections, malignancies, and acute rejection. A clinical assessment will be performed as noted in the Schedule of Assessments.

11.2 LABORATORY PARAMETERS

The following routine laboratory tests will be performed at baseline and/or as noted in the Schedule of Assessments:

Hematology:	hemoglobin, hematocrit, RBC count, WBC with differential, platelet count
Serum Chemistries:	BUN, creatinine, SGOT, SGPT, GGT, calcium,

	phosphorus, bilirubin (total and direct), total protein, albumin, glucose, alkaline phosphatase, LDH, uric acid, CO2, potassium, magnesium, sodium, and chloride
Fasting lipid profile:	Total cholesterol, LDL, HDL, HDL/LDL ratio, triglycerides
Pregnancy test (serum):	Serum pregnancy test must be performed prior to transplantation. Results must be obtained and documented prior to transplantation. Urine test is allowed in addition to serum test in patients where serum results are delayed. Follow-up tests should be performed in the event of secondary amenorrhea.

All laboratory analyses will be performed at the study institution's designated laboratory.

11.2.1 Procedures in the Event of Significant Abnormal Laboratory Values

Abnormal laboratory values considered clinically significant by the Investigator other than those associated with the patient's disease state, must be repeated as soon as possible and followed until they return to normal or an explanation is found. If a clear explanation is established, it should be recorded on the case report form.

11.3 OPPORTUNISTIC INFECTIONS

See Appendix B for classification criteria for opportunistic infections. Opportunistic infections during the study will be documented on a specific OI case report form and not on an Adverse Event case report form. Information captured on the OI case report form will include: date of onset, pathogen identified, and resolution, and action taken.

12 ADVERSE EVENTS

12.1 Baseline Medical Condition

It is not necessary to complete an Adverse Event (AE) page in the CRF for adverse medical conditions present during the screening period which do not worsen in either severity or frequency during the study. These conditions should be adequately documented in the patient's medical records, the CRF (i.e. previous medical conditions section) and any other medical documents.

Adverse medical conditions present during the screening period which become worse following exposure to study drugs should be reported as adverse events. They can also become Serious Adverse Events.

Any intervention performed during the study that corresponds to a condition during the screening period, should be recorded on the "Additional Observation" or "Concomitant Medication" pages of the CRF as appropriate.

12.2 DEFINITION OF ADVERSE EVENTS

12.2.1 Adverse Event (AE)

An adverse event (AE) is defined as any untoward or unfavorable medical occurrence associated with the use of a drug in humans, whether or not considered drug related (21 CFR 312.32(a)). An adverse event may include any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product (ICH E6, 1.2).

12.2.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the investigational drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity, or rate of occurrence that has been observed.

12.2.4 Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of the investigator, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

12.3 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version (CTCAE) (version 5.0 published Nov 27, 2017). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significant of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.
Grade 4 = Life-threatening consequences; urgent intervention indicated.
Grade 5 = Death related to AE.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Adverse events will be collected from the time of the first protocol mandated procedure until the study completion, or until 30 days after the subject prematurely withdraws from the study.

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will be determined by the site investigator and recorded on the appropriate AE eCRF. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in the table below

NCI-CTCAE attribution of adverse events

Code	Descriptor	Relationship
Unrelated Category		
1	Unrelated	The adverse event is clearly not related.
Related Categories		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.

3	Definite	The adverse event is clearly related

12.4 COLLECTION AND RECORDING OF ADVERSE EVENTS

12.4.1 Collection Period

Adverse events will be collected from the time of first protocol mandated procedure, until he/she completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject
- Questioning the subject in an objective manner
- Receiving an unsolicited complaint from the subject
- Clinical or laboratory evaluation, or medical records demonstrating pregnancy or overdose with or without an adverse drug reaction
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in section 11.3, Grading and Attribution of Adverse Events.

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously on the appropriate eCRF regardless of the relationship to study therapy regimen or study procedure.

All adverse events must be recorded by the site on the appropriate AE/SAE eCRF as outlined in the terms of the contract.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws or is withdrawn from the study, whichever occurs first. In summary, AEs/SAEs will be followed until they resolve or for 30 days after a subject is withdrawn or withdraws from the study.

12.4.4 Reporting of Serious Adverse Events

All investigators must report adverse events, including expedited reports, in a timely fashion to their respective IRBs in accordance with applicable regulations and guidelines. All adverse events will be reported to Sanofi as outlined in the terms of the contract.

12.5 FOLLOW-UP OF ADVERSE EVENTS

All adverse events must be documented and followed up until the event is either resolved or adequately explained, even after the patient has completed his/her trial treatment.

12.5.1 Planned Data and Safety Monitoring Reviews

The Data and Safety Monitoring Board will review safety data at least yearly during planned Data and Safety Monitoring Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The Data and Safety Monitors will be informed of an Expedited Safety Report in a timely manner.

12.5.2 Ad Hoc Data and Safety Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the Data and Safety Monitors may be called upon for ad hoc reviews per the request of the principal investigator.

13. MECHANISTIC ASSAYS

The objective of the mechanistic studies is to determine how ATG affects the immune system, both cellular and humoral, and, in particular, how ATG alters the immune response to the allograft during and after induction therapy. Mechanistic studies in the pre-transplant period will establish a baseline against which future laboratory results can be compared. The post-transplant mechanistic studies will elucidate the pathophysiologic and genetic mechanisms associated with the cellular and immune response against the allograft, the response to induction therapy, and graft rejection.

The immune cells and circulating antibodies referred to in secondary efficacy parameter (A) will be defined and measured as follows:

13.1 Blood for circulating antibodies

Blood for circulating antibodies will be tested for by standardized alloantibody testing by Luminex and control sera.[158] We will use these standardized methods to define kinetics, specificities and relative binding strength (mean fluorescence intensity, MFI) of DSA in each patient. Serum samples will be obtained at pre-transplant, 1, 3, 6, and 12 months post-transplantation. The HLA specificity, class I vs. II, and relative binding strength of the anti-HLA IgG will be determined using Luminex-platform single HLA class I and class II antigen microbead assays in combination with phycoerythrin (PE) secondary antibody conjugates for the detection of human IgG. To minimize any prozone effect (interfering factors which mask the antibody binding strength (MFI) serum samples will be diluted 1:8 according to established protocol to assess if there is an MFI increment after dilution. The Luminex single antigen beads will be analyzed using the LABScan™ 100 flow analyzer and an analysis program will assist in the assignment of the reaction strength (MFI). The presence and relative strength of non-HLA specific antibodies will be determined by ELISA and Luminex-platform assays. The final diagnosis of circulating antibodies will be made by an immunologist.

13.2 PRT ASSAY

The panel of reactive T-cells (PRT) assay consists of PBMC from transplant candidates pre-transplant being tested in IFN-gamma ELISPOT assays against a panel of HLA disparate B cell stimulator line. Results of the PRT have previously correlated with worse 12-month outcomes in kidney transplant recipients, and our goal here is to observe whether this is the case in heart transplant recipients, as well as whether ATG affects this correlation.

Regarding definition of a positive PRT, the optimal approach for quantifying and analyzing the results of the PRT assay, particularly for heart transplant recipients, remains unclear. We plan to use this opportunity to identify the optimal analytic approach. Our published data indicate that any stimulator that induces >25 IFNγ ELISPOTs per 300,000 responder cells represents a positive response to that stimulator, and that the percentage of positive responses (out of the total number of stimulators tested) correlates with risk of developing AR or lower GFR after kidney transplantation.[159,160] An alternative analytic strategy involves summing the responses to each stimulator and correlating the total (sum) with outcome.[158] The optimal number of stimulators to be included in the assay has not been formally determined, although our published data strongly indicate that 6 randomly selected distinct stimulator lines is sufficient.[161] We will define each result as either a) percentage of positive responses based on >25 or b) sum of the responses to each stimulator and calculate the results using all 10 stimulators. We will initially handle each variable as continuous (e.g. no. of positive ELISPOTs, no. of lines to which the responses are >25) and in a secondary analysis use ROC curves to define a potential threshold effect (e.g. responses to > 60% of stimulators or total responses > 400 ELISPOTs correlate with outcome). Depending on the results we will test alternative definitions of a positive result against a single stimulator (e.g. >50 or >100). We will also randomly and repeatedly analyze sets of responses from 4-10 stimulators from within the data to identify the lowest number of stimulators required to provide reliable correlations.[161]

13.3 LYMPHOCYTE ANALYSIS

Each blood sample from each subject at each time point (Pre- Transplant, Month 3 and Month 6 timepoints) will be analyzed by multiparameter flow cytometry to determine what proportion of the patient's immune cells are naïve, T effector, T memory, or B-cells. The immune cell populations will be identified by the following profiles:

(1) Allogeneic T-cells will be grouped as either CD4+ or CD8+ and will be further sub-classified as naïve, memory, or effector based on presence or absence of CD45RA, CD49a and CD62L.[162-164]
(2) T regulatory cells will be defined as CD3+, CD127(lo), CD4+, and CD25+ and Foxp3.[165,166] We will also include measurements of surface expressed and intracellular CTLA4, surface expressed (GITR) and intracellular pSTAT5 (among others) as these markers have been used by others as indirect correlates of T-reg function.[167]
(3) Accordingly CD8 suppressor cells will be defined as CD8+ CD3+ CD127(lo) and CD28-.[167]
(4) Dendritic cells or non-T-cell subsets will be defined as CD14-, CD56-, CD3-, CD19-.[168,169]
(5) B-cells will be defined as CD24+. Pre-pro-B cells will be defined as CD24- CD43+. Pro-B cells will be defined as CD24(int) CD43+. pre-B cells will be defined as CD24(hi) CD43-.

13.4 CYTOKINES

The production of pro-inflammatory markers (such as IL-1-beta, IL-2, IL-6, IL-17, TNF-alpha, IFN-gamma), and anti-inflammatory cytokines (such as IL-10, TGF-beta) will be measured pre-transplantation, then at 3 and 6 months. Post-transplant. These markers are important as they mediate the immune response in graft rejection and CAV. The cytokine milieu will give insight into the changes to the immune system from ATG induction therapy, its effectiveness, and the subsequent recovery of the immune system as the effects of ATG induction therapy dissipate.

14. STATISTICAL CONSIDERATIONS

14.1 SAMPLE SIZE CALCULATION

The study is intended to serve as a preliminary study. Comparative analyses will be primarily descriptive due to a small sample size, with an aim of 60 patients, randomized 1:1 to the treatment arm or control arm. The study is adequately powered to assess treatment efficacy for the composite primary endpoint only. The secondary parameters are for descriptive purposes only.

Sample size calculation for primary composite endpoint:

Assuming that the incidences of the composite primary endpoint are 40% in the control arm and 10% in the treatment arm, then under Fisher's exact conditional test for two proportions, a sample size of 60 detects a reduction in the composite primary endpoint in the treatment arm with a power of 70%.

14.2 ANALYSIS PLAN

14.2.1 Primary Efficacy Parameters

(A) Incidence of the primary composite endpoint of the development of de novo donor specific antibodies and ischemia on endomyocardial biopsy at 12 months post-transplant

14.2.2 Secondary Efficacy Parameters

To compare between groups:

(A) Changes in percentages of various subsets of immune cells at pre transplant, 3 months and 6 months. Changes in percentages of various circulating antibodies at pre-transplant, 1 month, 3 months, 6 months, and 12 months after transplant.
(B) Freedom from cardiac allograft vasculopathy (CAV) (defined as a change ≥0.5mm in maximal intimal thickness (MIT) of the coronary arteries by intravascular ultrasound at 12 months as compared to baseline)
(C) Change in coronary maximal intimal thickness intimal area, intimal volume, vessel area, intimal index, and percent atheroma volume at matched sites by intravascular ultrasound at 12 months

(D) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within first 12 months after transplantation
(E) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection episodes per patient within the first 12 months post-transplantation
(F) Freedom from acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection by the ISHLT biopsy grading scale in the first 12 months post-transplantation
(G) Freedom from biopsy proven cellular rejection ≥2R, biopsy proven antibody mediated rejection ≥AMR1 and any-treated rejection at 12 months post-transplantation
(H) Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection within the first 12 months
(I) Incidence of primary graft dysfunction (PGD) in the first 24 hours post-transplantation
(J) Patient and graft survival at 12 months post-transplantation
(K) The number of patients with both fatal and non-fatal infectious complications (especially CMV infection) within the first 12 months post-transplantation
(L) Freedom from the development of circulating antibodies within the first 12 months post-transplantation
(M) Average maintenance doses of Mycophenolate Mofetil, tacrolimus, and cumulative dose of corticosteroids at 12 months post-transplantation
(N) Average number of hospital days per patient, both during the transplant period and during the post-transplant period at 3 months, 6 months, and 1 year,
(O) Number of patients requiring hospitalization by 3 months, 6 months, or 12 months post-transplantation.
(P) Freedom from the development of circulating antibodies

14.2.3 Definition of Parameters

For both primary and secondary parameters, the 3 month assessment point will be defined as study day 90 $\pm$ 2 weeks, 6 month assessment point will be defined as study day 180 $\pm$ 2 weeks, 9 month assessment point will be defined as study day 270 $\pm$ 2 weeks and the 12 month assessment point will be defined as study day 360 $\pm$ 4 weeks.

For both the primary and secondary CAV parameters, the changes in maximal intimal thickness, averaged maximal intimal thicknesses at 10 matched sites per patient, intimal area, intimal volume, vessel area, intimal index, and percent atheroma volume at the 12 month assessment point will be used for analysis.

Patients with infectious complications will include:

(1) Patients with viral infections, proven by positive serology and symptoms consistent with the suspected viral infection
(2) Patients with documented bacterial/ opportunistic infections

The definition of rejection will be as follows:

Patients with acute rejection will include:

(1) Patients with acute cellular rejection that is biopsy proven with ISHLT grade 2R or worse histology
(2) Patients with acute antibody-mediated rejection that is biopsy proven with immunohistochemistry stains positive and concomitant significant hemodynamic compromise
(3) Patients with hemodynamic compromise who are treated for acute cellular and/or antibody-mediated rejection, regardless of whether or not a biopsy is done and regardless of histologic findings if a biopsy is done
(4) Patients who die within 12 months of transplantation before experiencing a primary end-point rejection
(5) Patients who are lost to follow-up within 12 months after transplantation and have not experienced a primary end-point rejection

A rejection episode begins with a positive biopsy or with the start date of treatment for rejection, whichever comes first. Resolution of rejection will be defined when no further treatment is required. If continued treatment is required for the current rejection (according to the regimen specified in Table 1), the rejection is still considered an ongoing episode. A second episode of rejection may not be counted until the first episode has resolved. Follow-up biopsies should occur within 2 weeks (with an allowable window of 10 days to 21 days) after the end of treatment for the rejection.

Time to first acute cellular, antibody-mediated, hemodynamic compromise, and any-treated rejection will be calculated from the date of transplantation to the date the patient first experiences acute rejection, date of death, date of re-transplantation, date of graft loss, or date of premature drop-out (for patients who are lost to follow-up), whichever comes first (see section 2.2.1 for definition of rejection). If a patient never experiences any of these events while being followed, he/she will be censored at the last follow-up.

Re-transplantation will be calculated form the date of original transplantation to the date of re-transplantation or date of death, whichever comes first. If a patient does not have a re-transplantation or does not die, he/she will be censored at the last date of follow-up.

Death will be calculated form the date of start of therapy to the date of death. If a patient does not die while being followed, he/she will be censored at the last date he/she is known to be alive.

14.2.4 Statistical and Analytical Methods

A descriptive analysis of the endpoints will be applied owing to the small sample size. This is a multi-center pilot study and not designed, nor intended to provide data on the statistical significance of endpoints.

14.2.5 Intent to Treat Population Analysis

All patients randomized in the study will be involved in the statistical analyses. The primary analysis population will be the intent-to-treat population.

14.3 SAFETY DATA ANALYSIS

The safety population includes all patients who received at least one dose of drug (either Thymoglobulin® or No Induction or Control) and for whom at least one post-baseline safety assessment was made.

The safety parameters include clinical adverse events, malignancies, opportunistic infections, laboratory tests, and vital signs. Incidence of clinical adverse events will be calculated overall, by body system, and by adverse event. Incidence will also be presented by relationship to trial medication and severity.

14.4 FINAL STUDY ANALYSIS

A final study analysis will be prepared demonstrating descriptive statistics of the primary and secondary endpoints.

15. SOURCE DATA

15.1 SOURCE DATA

All study data should be verifiable by source documentation. Most often this will be present in the patient's medical record. Except in rare instances, study documents (CRFs and data collection tools) are not source documents. "Shadow charts" – duplications of material from the medical record are not source documents.

15.2 ACCESS TO SOURCE DATA

The responsibilities of the investigator require that health authorities have access to (and may when required by applicable law copy) source documents. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals.

16. QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. An internal auditor will regularly review the conduct of the trial, verify adherence to the protocol, and confirm the completeness, consistency, and accuracy of all documented data.

16.1 DATA HANDLING

The investigator is required to ensure that all CRFs are completed for every patient entered in the trial. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded with an audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

17. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

17.1 STATEMENT OF COMPLIANCE

The clinical study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in the study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate IRB. Any amendments to the protocol or the consent materials must also be approved before they are implemented.

An IND exemption will be requested from the FDA for this protocol.

17.2 INFORMED CONSENT

The consent process provides information about the study to a prospective participant and allows adequate time for review and discussion prior to their decision. The principal investigator or designee will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) must read, sign, and date a consent form before undergoing any study procedures. Consent materials must be presented in participants' primary language or a short form may be used. A copy of the signed consent form must be given to the participant.

The consent process is ongoing. The consent form must be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

17.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information.

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APPENDIX A

A-1 Standardized ISHLT Cardiac Biopsy Grading

GRADE	ACUTE CELLULAR REJECTION (2004)
0	No rejection
1R	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
2R	Two or more foci of infiltrate with associated myocyte damage
3R	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis

GRADE	ANTIBODY MEDIATED REJECTION (2013)
pAMR 0: Negative for pathological AMR	Both histological and immunopathologic studies are negative
pAMR 1 (H+): Histopathologic AMR alone	Histological findings present and immunopathologic findings negative
pAMR1 (I+): Immunopathologic AMR alone	Histological findings negative and immunopathologic findings positive
pAMR 2: Pathological AMR	Both histological and immunopathologic findings are present
pAMR 3: Severe pathological AMR	Severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis and/or karyorrhexis, and marked edema

A-2 Acute Rejection—Diagnosis and Treatment

ISHLT BIOPSY GRADE	NO HEMODYNAMIC COMPROMISE PRESENT	HEMODYNAMIC COMPROMISE PRESENT
1R	No treatment	High dose IV 500 mg corticosteroids for 3-10 days OR 5 days of ATG 1.5 mg/kg
2R	500 mg IV corticosteroids for 3 days followed by immediate return to baseline	High dose IV 500 mg corticosteroids for 3-10 days ± 5-10 day taper to baseline AND 5 days of ATG 1.5 mg/kg

ISHLT BIOPSY GRADE	NO HEMODYNAMIC COMPROMISE PRESENT	HEMODYNAMIC COMPROMISE PRESENT
3R	High dose IV 500 mg corticosteroids with 5-10 day taper to baseline and 5 days of ATG 1.5 mg/kg	High dose IV 500 mg corticosteroids with 5-10 day taper to baseline and 5 days of ATG 1.5 mg/kg

APPENDIX B

CLASSIFICATION OF OPPORTUNISTIC INFECTION

A. CMV

CMV disease is defined as CMV infection supported by virologic evidence of active CMV replication by either viral culture (including shell-vial technique), or CMV antigenemia assay, or CMV PCR assay along with one of the following:

CMV syndrome: defined as episodic fever and no response to antibiotics if used (i.e., two spikes of fever greater than 38 C during the 48 hours when antibiotics are being used); plus one of the following:
• malaise
• fall in neutrophil count over three consecutive daily measurements
OR
CMV hepatitis: Defined as evidence of CMV in the liver confirmed by liver biopsy. The biopsy must be characterized by one of more of the following:
• Presence of cells with positive immunostaining or immunofluorescence or in situ hybridization for CMV or CMV inclusions
• Histological evidence of CMV hepatitis
In addition, at least one liver function test value (AST, alkaline phosphatase, or bilirubin) must be significantly outside the normal range.
OR
CMV gastroenteritis, esophagitis, or colitis confirmed by biopsy.
The biopsy must be characterized by the presence of cells with positive immunostaining or immunofluorescence or in situ hybridization for CMV or CMV inclusions.
And, either of the following must be present:
a) signs and symptoms of upper gastrointestinal tract infection
including: nausea, vomiting, anorexia, dysphagia, abdominal pain or
b) cramping, signs, and symptoms of colitis including persistent diarrhea, cramping or abdominal pain.
OR
CMV disease with involvement of the lung confirmed by Broncho-alveolar lavage (BAL) characterized by the following:
• Presence of cells with positive immunostaining or immunofluorescence or in situ hybridization for CMV or CMV inclusions or positive viral culture.
In addition, at least two of the following must be present:
a) dyspnea
b) interstitial infiltrates on chest x-ray
c) requirement for supplemental oxygen and/or ventilator assistance or decreased pO2 (<80 mmHg) with an increased A-a gradient (i.e., >20).
OR
CMV retinitis in one or both eyes based on dilated fundus exam by an ophthalmologist experienced in the diagnosis of CMV retinitis

B. Aspergillus:
1. Sputum
2. Pulmonary or sinus invasive disease
3. Disseminated or metastatic disease

C. Candida:
    1. Urinary tract infection
    2. Mucocutaneous (including oral, esophageal, rectal, and vaginal)
    3. Invasive tissue disease
    4. Fungemia/disseminated disease

D. Pneumocystis:
    1. Pulmonary disease

E. Cryptococcus:
    1. Crytococcosis

F. Listeria:
    1. Listeriosis

G. Herpes zoster:
    1. Localized (1-2 dermatomes)
    2. Disseminated cutaneous disease
    3. Visceral disease

H. Herpes simplex:
    1. Herpes simplex

SCHEDULE OF ASSESSMENTS

	Screening /Baseline[1]	Day of[3] Htx	Visits Timeline																
STUDY DAY	0	1	2	3	4	5	7	14	21	28	42	56	90[6]	120[6]	150[6]	180[6]	240[6]	300[6]	365[6]
STUDY WEEK/MONTH	0	W1						W2	W3	W4	W6	W8	M3	M4	M5	M6	M8	M10	M12
VISIT NUMBER	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ASSESSMENT VISITS																			
Medical History	X																		
Informed Consent	X																		
Randomization[2]	X																		
Clinical Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunosuppressant Therapy		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration		X[5]	X[5]	X[5]	X[5]	X[5]													
Pre-Treatment Medications		X	X	X	X	X													
Antimicrobial Prophylaxis		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fungal prophylaxis		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
CMV Prophylaxis		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Corticosteroids		X	X	X	X	X	X	X	X	X	X	X	X	X	X				
HMG CoA Reductase Inhibitor		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin C, E		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Aspirin		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAFETY																			
Vital Signs/Body weight		X[5]	X[5]	X[5]	X[5]	X[5]	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Opportunistic Infections		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EFFICACY																			

	Screening/Baseline[1]	Day of[2] Htx	Visits Timeline																
STUDY DAY	0	1	2	3	4	5	7	14	21	28	42	56	90[6]	120[6]	150[6]	180[6]	240[6]	300[6]	365[6]
STUDY WEEK/MONTH	0	W1						W2	W3	W4	W6	W8	M3	M4	M5	M6	M8	M10	M12
VISIT NUMBER	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Data Collection Rejection		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Echocardiogram[4]								X		X		X	X			X			X
Endomyocardial Biopsy[4]								X		X		X	X			X			X
Intravascular Ultrasound[4]											X								X[7]
Data Collection Patient/Organ Survival		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LABORATORY TESTS																			
Hematology (CBC with Diff) [4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Serum Chemistry[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Lipid Profile[9]	X												X			X			
Blood Loss		X	X	X	X	X													
Serum Pregnancy Test (female)	X																		
Trough levels (Tacrolimus) [4]			X	X	X	X	X	X	X	X	X	X	X[7]	X[7]	X[7]	X[7]			X[7]
HLA Donor Mismatch	X								X				X			X			X
Cytomegalovirus Status (recipient and donor)	X																		
Epstein-Barr Virus Status (recipient and donor)	X																		
Circulating Antibodies (PRA)	X									X			X			X			X[7]

Mechanistic[8] Assessments	X												X[8]			X[8]			

[1] Screening and consenting – eligible subjects will be enrolled in the study while on the UNOS waitlist. Screening labs may be drawn after consent obtained and at any time prior to heart transplantation.

[2] Patients consented to the study and meeting study entry and randomization inclusion and exclusion criteria will be randomized in a 1:1 ratio to either Thymoglobulin® or No Induction or Control during the immediate post transplantation period.

[3]Day of Heart Transplant

[4]For subjects repatriated to Kaiser 60 days after heart transplant, to be performed per KP standard of care protocol.

[5] The first 2 doses (post op Day 0 and 1) will be infused over 8 hours. Vital signs every 15 minutes x 1 hour, then every 30 minutes x 2 hours, then every hour for the remaining infusion period. Once infusions are completed, return to routine vital signs monitoring frequency**.** subsequent infusions (Days 3,4 &5) will be over a minimum period of 4-8 hours.

[5] Starting with 3rd dose vital signs every hour during infusion.

[6] Study visits and procedures will be conducted at Kaiser locations per standard of care for patients that return their care back to Kaiser Permanente

[8].Kaiser patients will have blood drawn at their Kaiser location and the blood sample(s) will be shipped to Cedars-Sinai for the mechanistic assessments

[9]These laboratory tests will be collected as standard of care as required and as needed. If these labs are available, the data will be collected. However, if the labs are not collected as standard of care, this would not be considered a protocol deviation.